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AGE AND TISSUE RESPIRATION[1]

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It has been found that the basal metabolism in at least two species of bird [pigeon (22) and fowl (19)] and three mammals [man, (17), (1), (6), (15), (2), (3), (21), (14), (24), hog (9), (25) and calf (7)] decreases, although not at a constant rate, as the animal grows older and that this decrease while much less in extent continues throughout maturity when growth is stationary. In two other species [rat (18), (4), (12), (8) and dog (16)] the initial decline from the metabolic peak of early youth is followed by a steady constant rate during adult life and into old age. Victor and Potter (23) have found a significant decrease in the oxygen consumption of excised lymph nodes of 5 genetically pure strains of mice aged 6 to 10 months as compared to those aged 6 to 8 weeks. The present work was undertaken to determine whether a comparable difference in metabolic activity existed for other tissues removed from young and old animals, and if such a change occurs, in what tissues it reaches its greatest extent.

METHODS. The O_2 consumption of liver, kidney and ventricular cardiac muscle of albino mice of a genetically pure strain (Bagg albino) was studied at two different age periods. This strain has been inbred by brother and sister matings since 1922. Its characteristics are: genetic determinant for dark-eyed, brown agouti; normal eyed, eared, tailed and footed, without behavior habits; large litters. The animals were obtained through the courtesy of the Department of Genetics of the Carnegie Institution of Washington at Cold Spring Harbor, New York. The measurements were made in differential volumeters similar to those devised by Fenn (10). In the case of the liver and kidney the experimental tissues were cut with a razor blade while immersed in Ringer's solution into slices of approximately 0.3 mm. thickness. The cardiac muscle

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was prepared by cutting off the two auricles and opening each ventricle by a single incision through the wall from the auriculo-ventricular valve to the apex of the heart, leaving the right and left sides still connected by the intact interventricular septum. All blood was washed out. It was found that by this method a higher and more constant rate of O_2 consumption was obtained than when either the intact whole heart or the finely sliced ventricular muscle was used. Variation due to differing degrees of work by the heart was avoided by the inhibition to contraction brought about by removal of the auricles and section of the ventricles. The quantity of tissue used varied between 70 and 150 mgm. for the heart, between 60 and 90 mgm. for the liver, and between 20 and 40 mgm. for the kidney, all being moist weights determined to 0.2 mgm. on a Roller-Smith torsion balance, after blotting with filter paper to remove excess fluid. During an experiment the cardiac muscle was immersed in 1 cc. of phosphate buffered Ringer's solution, the liver and kidney in 1 cc. of unbuffered Ringer's containing 0.2 per cent glucose. The Ringer's solution consisted of 0.9 per cent NaCl, 0.022 per cent KCl, and 0.0236 per cent $CaCl_2$. The phosphate buffer was adjusted to pH 7.44 and contained 10 mgm. of P per 100 cc. The respirometer vessels containing solution and tissue were flushed with pure O_2 for 5 minutes and put into the bath within 30 to 40 minutes of the time the animals were sacrificed. The temperature of the water was 37.5°C. $\pm$ 0.05. The respirometers were shaken 180 times per minute through an arc of approximately 4 cm. Readings were taken at approximately 10 minute intervals over a period of 1 to 3 hours. The rate of O_2 consumption almost always remained constant for the first hour after an initial short period of equilibration, and usually, but not invariably, declined slowly through the second hour. The rate was taken to be that of the constant period.

In all cases histological sections were made of the tissues after being in the respirometer to rule out any anatomical or pathological abnormality. In addition, complete autopsies, including histologic examination of lungs, spleen, pancreas, adrenals, and testes, or uterus and ovaries as well as the organs used in the determinations were performed on all mice and any animals containing significant pathologic processes were discarded.

Animals of two age groups were used, the younger between 4 and 9 weeks, the older between 50 and 60 weeks. In both groups about $\frac{2}{3}$ of the animals were females. The experiments were performed during the winter and spring. Differences due to possible seasonal variation in metabolism were avoided by so timing the determinations that approximately equal numbers of each group were used at the same period of the year.

RESULTS. Figures 1, 2 and 3 are scatter charts showing each individual experiment for heart, liver and kidney tissue respectively, the deviation

of each determination from the mean and the amount of overlapping of the two age groups. It is obvious that the values for heart and liver in

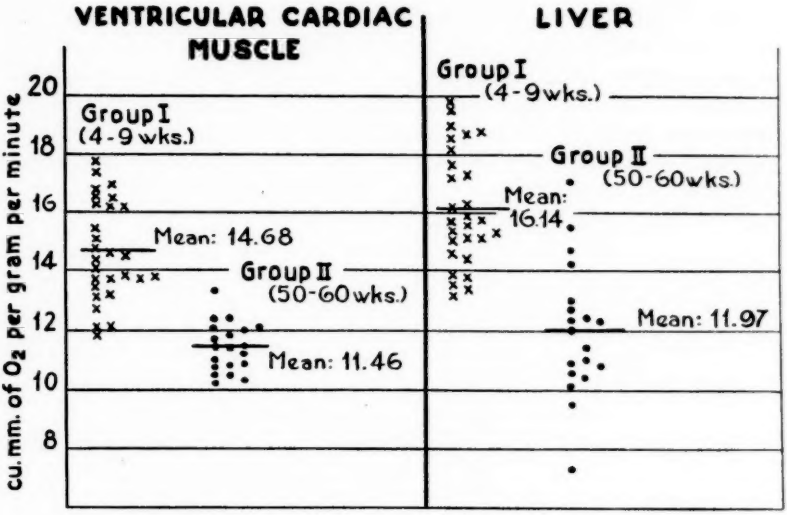

Fig. 1

Fig. 2

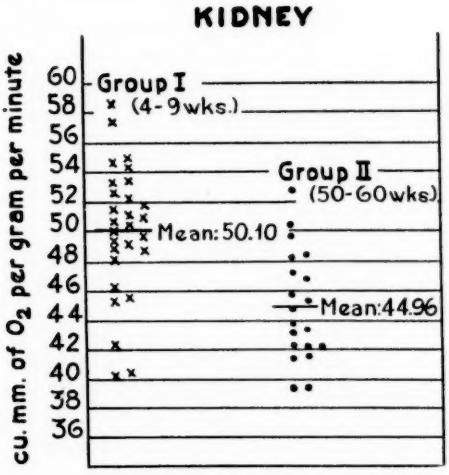

Fig. 3

each age group fall much closer to their respective means and that likewise there is less overlapping than is the case with kidney. However, the

rates of O_2 consumption for the latter tissue are so much higher than are those for the two former that the coefficients of variability are actually slightly less for the kidney than for all the others except the ventricular muscle in the old group. This is shown in table 1, which is a summary of the experiments, giving for each group the mean value for O_2 consumption in cubic millimeters per gram of moist tissue per minute, the probable error of the mean, the standard deviation, and the coefficient of variability. Table 2 shows the difference in means, the probable error of the difference, the ratio of the difference to the probable error, and the percentage de-

TABLE 1*

	NO. OF DETERMINATIONS	HEART			LIVER			KIDNEY		
		Mean	Standard deviation	Coef. of variability	Mean	Standard deviation	Coef. of variability	Mean	Standard deviation	Coef. of variability
				per cent			*per cent*			*per cent*
Group I (4–9 wks.).....	28	14.68 ±0.21	1.64	11.20	16.14 ±0.24	1.90	11.8	50.10 ±0.55	4.47	8.92
Group II (50–60 wks.)..	20	11.46 ±0.11	0.78	6.76	11.97 ±0.32	2.14	17.9	44.96 ±0.55	3.66	8.15

TABLE 2*

	HEART	LIVER	KIDNEY
Diff. of means of group I and group II.	3.22 ± 0.24	4.17 ± 0.40	5.14 ± 0.78
Diff. of means / P.E. diff. of means	13.60	10.43	6.64
Percentage decrease in group II as compared to group I.....	28.05	34.8	11.45

* Figures in terms of cubic millimeters of O_2 per gram (moist weight) per minute.

crease in the rate of O_2 consumption in the older group. The differences are statistically significant, being from 6.64 to 13.60 times the probable errors of the differences.

It is interesting that although the greatest numerical decrease in the rate of O_2 consumption occurs in the kidney this organ because of its much greater total consumption has the smallest percentage decrease, while the liver with a relatively much smaller total consumption and a slightly smaller numerical decrease has a percentage decrease over 3 times that of the kidney. The heart is intermediate. If these relations hold

for the intact animal, taking the weight of the liver as 2.2 grams, of the two kidneys as 0.49 gram, and of the ventricles of the heart as 0.15 gram, the total O_2 consumption per minute of each respectively would be 9.67 mm^3., 2.52 mm^3., and 0.48 mm^3. The 34.8 per cent decrease in O_2 consumption of the liver would then amount to 3.37 mm^3., while the smaller percentage decrease in that of the kidneys and ventricles could be only 0.29 mm^3. and 0.14 mm^3. The change in the metabolic activity of the liver, then, would account for almost eight times as much of the decrease in total O_2 consumption of the mouse as would the changes in the other two organs together, and would be responsible for a relatively large proportion of the decline in O_2 consumption of the animal body with increasing age.

Hawkins (11) has found that the QO_2 of excised liver tissue of rats aged 3 to 21 days is 13.2 as compared to 9.4 for rats aged 12 months, a change similar to that shown here. However, at 22 months it was 11.5. No mention is made of the degree of genetic identity of his rats. Differences in strains of the groups compared may account for metabolic variation, a fact stressed by Victor and Potter (23). In addition it is not stated whether statistical analysis has shown the figures to be significant. It must be remembered that the two age periods compared in the present paper represent immature partially grown mice and mice in the mid-period of their maturity. As yet no animals in late maturity or senility are available. A somewhat more distant parallel is the decrease in O_2 consumption with age of tissues grown in culture, of embryonic heart, and of chorion, reported by Warburg and Kubowitz (24). Murray (20) has shown a tremendous decrease in O_2 consumption of the whole chick embryo between the 6th and 18th day of incubation as measured in a Warburg volumeter. A decrease in the respiratory rate of growing plants has likewise been demonstrated (13).

SUMMARY AND CONCLUSIONS

The oxygen consumption of excised liver, kidney, and resting ventricular cardiac muscle of a genetically pure strain of mice was studied at two age periods, 4 to 9 weeks and 50 to 60 weeks.

The rate of oxygen consumption of all three tissues in the older group was significantly less than that in the younger group.

The greatest percentage decrease in rate of O_2 consumption of the older group as compared with the younger occurred in the liver (34.8 per cent). The kidney showed the least percentage decrease (11.5 per cent) while cardiac muscle was intermediate (28 per cent).

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MUSCLE TONUS, INTRAMUSCULAR PRESSURE AND THE VENOPRESSOR MECHANISM

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A method for the measurement of muscle tonus is greatly needed. Neither the knee jerk (1) nor the hardness of the surface of a muscle (2) has afforded a practical means. Probably the oxygen consumption and carbon dioxide production of the body are to a large extent expressions of general muscle tonus.

It occurred to us that the longitudinal pull exerted by a tonic muscle must induce a transverse pressure between the fibres of the muscle; and that this intramuscular pressure might be measured by the pressure required to inject a small amount of a saline solution into the body of the muscle through a hypodermic needle. This method has in fact proved practicable and has yielded the results to be here reported.

Intramuscular pressure and the venous return. Our interest in measurements of intramuscular pressure is particularly concerned with the influence that this pressure must have upon the venous return to the heart (3). The tonus influencing the circulation of the blood is, as we shall show, not merely the tonus of the blood vessels—arteries, capillaries and veins—but in addition also the tonus of the tissues and organs through which the vessels pass. So long as tonus maintains a pressure within the tissues, the blood flowing from the arteries into the tissue capillaries cannot lose its pressure, or vis a tergo, below that of the tissues. The pressure retained by the blood in the capillaries is the force that drives the blood onward into the veins. When, on the contrary, in illness or after surgical operations tonus is considerably diminished, the blood coming from the arteries may lose nearly all of its pressure in the capillaries and stagnate in the tissues (4).

It is well recognized that, during physical exercise, every contraction of a muscle squeezes blood into the veins and toward the right heart. But that force is kinetic. Quiescent, but tonic, muscles, on the contrary, by their elastic pressure, merely give back some of the energy that they have received. Their degree of tonic elasticity determines whether much

or little of the force of arterial pressure is transferred to the venous stream.

It has long been recognized that intra-abdominal pressure (5), the pressure upon the viscera due to the tonus of the diaphragm and the muscles of the body walls, affords an important support to the circulation. But this support is only a small part of that afforded by the tonus of the body as a whole. The evidence here presented indicates that the tonic pressure within the tissues is essential to the maintenance of the venous return of blood to the heart.

The Riml experiment. This conception of the relation of general body tonus to the venous return was suggested to us in part by an experiment reported by Riml (6), although Riml himself did not so interpret it, and in part by observations on the diminished tonus of the respiratory muscles in men after voluntary over-breathing and after surgical operations (7). Before describing our method of measuring intramuscular pressure we will first report our confirmation and extension of Riml's observations.

For this experiment we have used four cats. Three of them were normal, and one had been completely sympathectomized by Dr. W. B. Cannon, who generously presented it to us. In each of these experiments the animal was narcotized with nembutal: 40 mgm. per kilo injected intraperitoneally in a 5 per cent solution. Complete anesthesia resulted, but with retention of nearly normal tonus as indicated by very active knee jerks. After tracheotomy, the thorax was opened under artificial respiration. The pulmonary artery was then suddenly clamped; a trocar was thrust into the right auricle; and the outgushing blood was collected and measured. The operation of clamping the pulmonary artery and inserting the trocar required only two or three seconds and was completed before there was time for any considerable degree of asphyxia to develop. The absence of asphyxia was evidenced by the fact that the color of the outflowing blood was as bright as normal venous blood.

The weights of the animals, the volumes of the outflowing blood and the rapidity of the outflow are shown in table 1.

The first gush of blood came with a sufficient force to raise the column in a vertical tube 10 or 12 cm., as Riml also found. Evidently, even after the heart action is eliminated by occlusion of the pulmonary artery, there is still in the tissues a pressure sufficient to drive more than half of all the blood in the body to the right heart, and a quarter of it in a few seconds. The amount of the pressure developed in this experiment is virtually the same as that occurring throughout the entire vascular system when the heart is brought to a rather prolonged standstill by stimulation of the vagus. Arterial pressure then falls, while venous pressure rises. At any time in life only a small part of the blood is contained in the arteries and only about twice as much in the veins; by far the greater part is in the capillaries of the tissues. When the heart action is stopped, either by

vagus stimulation or by clamping the pulmonary artery, the pressure throughout the entire vascular system tends immediately to become uniform: the same pressure alike in arteries, veins and capillaries. And the amount of this pressure is approximately that previously existing in the large blood reservoirs of the body: the tissue capillaries.

It is a significant fact that the capacity to produce the Riml phenomenon is lost within about 10 minutes after death. Opening the right heart then reveals no considerable pressure and only a slow outflow of blood. The reason for the failure of pressure and outflow lies in the loss of general body tonus that occurs in this time. In support of this statement we would report observations (7) made by one of the authors some 20 odd years ago when, as a member of the First Resuscitation Commission, he undertook to determine, partly upon cats, the comparative effectiveness of various forms of manual artificial respiration. It was found that the re-expansion of the chest after manual compression is dependent upon the tonus of the

TABLE 1

Showing the volume of blood squeezed out of the tissues by their tonic elasticity

CAT	WEIGHT IN KILOS	TIME IN SECONDS					TOTAL OUTFLOW AS PER CENT OF BODY WEIGHT
		15	30	45	60	180	
		Outflow of blood in cc.					
I Normal	1.57	33	40	50	52	56	3.5
II Normal	3.02	60	75	88	102	107	3.5
III Normal	2.45	47	60	72	79	85	3.4
IV Sympathectomized	2.35	40	54	64	68	72	3.0

respiratory muscles. Within 10 minutes after stoppage of the heart the elastic recoil of these muscles ceases. Thereafter no air is drawn in; for the chest does not re-expand. Tonus is gone; and with the abolition of tonus, not only is the positive pressure in the tissues abolished, but also a large part of the negative pressure of the thorax. Thus the total effective venous pressure is doubly diminished.

This pressure, under which the blood gushes out in the Riml experiment, is not due to asphyxia. Nor is it due to a general vasomotor reaction; for in one of the cats the entire sympathetic nervous system had been removed. The pressure may be, and probably is, partially due to the elasticity of the walls of the capillaries themselves (8). But if that elasticity were the prime factor, the pressure would not disappear coincidentally with the disappearance of general body tonus. Muscles and blood vessels withstand asphyxia quite well for fairly long periods, but the central nervous system fails within 10 minutes. And it is from the central nervous system that come the impulses inducing tonus in the skeletal

muscles. When these impulses over the motor nerves cease, the tonus of the muscles fails; and the pressure upon the blood in their capillaries is abolished.

Measurement of intramuscular pressure. For the measurement of intramuscular pressure in man we have chosen to use a muscle in a condition of relaxation, i.e., a low degree of tonus. The subject lies upon his back with his left elbow flexed at an angle of about 135° and the intramuscular pressure is determined in the left biceps as thus relaxed.

If, instead of this partial relaxation the biceps is subjected to even a slight tension, as it is when the elbow is extended, the active stretch and the tonus that stretching induces produce a much higher intramuscular pressure than is found in the relaxed muscle. We state this point immediately and emphatically for we do not wish to be understood as implying that muscle tonus is a quantity in which at any one time all the muscles participate to an equal degree. Tonus is not like blood pressure, which is nearly the same in all large arteries. The tonus and intramuscular pressure throughout the body as a whole are not only variable, but in general are certainly much higher than the values found in the relaxed biceps. But in order to determine the variations of tonus under different general conditions we have deliberately chosen to make the measurements on a muscle in which the conditions applying to that particular muscle are constant and induce only a low degree of tonus.

The hypodermic needle that is inserted into the muscle is a number 20. The opening at the end of the needle is closed with solder, and three or four holes have been drilled or ground in the side of the needle near its point. Into the butt end of the needle is inserted a piece of capillary glass tubing of 1 mm. bore and 5 cm. length; it is ground to fit tightly in the needle and has a number of transverse scratches on its surface by which even the slightest movement of the meniscus within the capillary can be estimated. For measuring the pressure a U-tube manometer is employed. It is graduated in millimeters and filled with water to any desired pressure by means of a rubber bulb attached at the bend of the manometer and compressed by a thumb screw.

The skin over the biceps is sterilized with alcohol and rendered anesthetic with novocaine. The needle to be used for the pressure determinations is sterilized, filled with sterile saline and inserted into the center of the muscle. The glass capillary tube is inserted in the butt of the needle and is then filled to about two-thirds of its length with saline by means of a small hypodermic syringe with a needle fine enough to be inserted into the capillary. This degree of filling leaves a visible meniscus in the capillary. The free end of the capillary tube is then connected to the manometer by means of a sterilized rubber tube containing air.

A few cubic millimeters of saline are first injected into the muscle to

make sure that there is no mechanical obstruction. Then, starting at zero, one observer gradually raises the pressure in the manometer, while a second observer watches the meniscus until it starts to move. He then signals to the first observer who reads the pressure on the manometer. The amount of fluid injected at each observation is not more than one or two cubic millimeters. Larger amounts injected slowly afford no clear cut indication of the intramuscular pressure. They are too rapidly absorbed. It is the first movement of the meniscus in the capillary glass tube that must be taken as the criterion for reading the pressure on the manometer.

TABLE 2

Showing the intramuscular pressure in millimeters of water in 10 healthy young men and 10 patients in bed in the surgical ward

	I	II	III	IV	V	VI	VII	VIII	IX	X	AVERAGES
Healthy young men.....	79	64	73	83	59	75	60	80	75	90	74
Patients in bed.....	54	50	61	21	52	55	50	27	54	50	47

TABLE 3

Showing some measurements of intramuscular pressure on men before and after surgical operation

12 HOURS BEFORE OPERATION	HOURS AFTER OPERATION	INTRAMUSCULAR PRESSURE	NOTES
104	½	56	Herniotomy, local anesthesia
56	3	38	Resection of colon, general anesthesia
97	24	58	Resection of rectum. General anesthesia
62	4	40	Gastrectomy. General anesthesia
77	24	61	Hernia operation. Local anesthesia
65	6	30	Gall bladder. General anesthesia
64	48	52	Thyroidectomy. General anesthesia
Av...73		48	

With this method we have made observations on 63 men; about one-third were students and internes in normal health; the others were hospital patients. The observations were in the nature of a general survey. We are here concerned with their physiological rather than their clinical relations, and we need only report therefore the general bearing of the evidence. This is shown in table 2 in which typical observations on 10 healthy young men and 10 patients awaiting operation in the surgical wards of the New Haven Hospital are reported.

Modern anesthetic and surgical methods have rendered severe degrees of surgical shock rare in modern hospitals. But it is probable that even

now few patients return to their beds after any considerable operation without some decrease of vitality. In table 3 are shown some observations on the corresponding decrease of intramuscular pressure.

On animals undergoing operative procedures in the laboratory we have found a progressive decrease of intramuscular pressure down almost to zero when the animals are moribund.

Strychnine as a "cardiac stimulant." The conception of muscle tonus as a factor in the circulation may explain why surgeons a generation ago considered strychnine as a heart stimulant; whereas strychnine is in fact an almost neutral drug when tested on an excised heart under experimental conditions. We therefore administered 1/20 of a grain of strychnine sulphate to a patient 8 hours after a surgical operation; and as controls the same dose was given to two healthy young men. The injections of the

TABLE 4

Showing the influence of strychnine on intramuscular pressure in two healthy men (D and H) and in a patient some hours after operation

TIME, MIN.	SUBJECTS			NOTES
	D	H	X	
0	90	85	68	Pressures in mm. water
5				1/10 grain strychnine sulphate injected into right biceps
10	142	130	76	
15	154	154	95	
20	159	163		
25	142	141		

drug were made into the right biceps; the measurements of intramuscular pressure were made in the left biceps.

In table 4 are shown the effects upon intramuscular pressure. They afford clear evidence that strychnine may tend to support the ciruclation by increase of the tonic intramuscular pressure.

The influence of carbon dioxide on intramuscular pressure. The influences exerted by acapnia and by hypercapnia upon the circulation have been the subject of much investigation, and almost as much dispute. Henderson (9) was first led to recognize the existence of the venopressor mechanism as a factor distinct from the vasomotor system by observing that under excessive pulmonary ventilation the circulation of the blood is diminished. He found, in particular, that under maximal artificial respiration, after the thorax is opened, the circulation rapidly fails. The failure is not in the heart, nor in the vasomotor control of arteries or veins, but rather in the decreasing volume of blood returning from the tissues to the right heart.

The venopressor mechanism thus discovered remained nevertheless for many years very ill defined (10). The observations of Henderson and Harvey (11) indicate that in so far as carbon dioxide is a factor in this mechanism the influence of this hormone may be exerted in part upon the muscles themselves and in part upon the spinal centers of the motor nerves; for acapnia and hypercapnia induce effects upon the venous return even in decapitated animals. It appears to us probable that in addition the respiratory center influences not only the tonus of the respiratory muscles but to some degree also that of all the other muscles of the body.

The effects of forced breathing for two minutes and of inhalation of 7 per cent carbon dioxide for five minutes on the intramuscular pressure in the left biceps were tested on four men. The results, as given in table 5, show a distinct acapnial decrease, and hypercapnial increase in all cases (12).

Intramuscular pressure under maximal muscular contraction. The idea that mere tonus induces a sufficient pressure in the musculature of the

TABLE 5

Showing the influence of forced breathing and the inhalation of carbon dioxide on intramuscular pressure

SUBJECTS	INTRAMUSCULAR PRESSURE (MM. WATER)		
	Normal	After forced breathing	After CO_2 inhalation
H	97	64	115
K	95	77	128
M	124	79	158
S	94	74	120

body to be a prime factor in maintaining the circulation is, we believe, original with the authors of this paper. But the closely related idea that vigorously contracting muscles produce an internal pressure is as old as William Harvey and Borelli (13). It occurred to us that it would be of interest to determine whether the maximal intramuscular pressure would be sufficient to stop the circulation through a muscle. Such would be the case if the intramuscular pressure exceeded arterial pressure (14).

On this point we have only one experiment to report but it was decisive. A young man of good muscular development was the subject. His arterial pressures were 120/80 mm. of mercury. When standing at ease with the gastrocnemius moderately relaxed, the internal pressure of that muscle was 143 mm. of water. When he stood on one toe, so that the whole weight of his body was carried by the gastrocnemius, the intramuscular presssure was 312 mm. As 312 mm. of water is only 24 mm. of mercury, or only about a third of diastolic arterial pressure, the circulation through the muscle was certainly not stopped.

Somewhat to our surprise we have found no increase of tonic intramuscular pressure after moderate physical exercise.

CONCLUSIONS

A technique for determining the pressure within a muscle is described, and is used to show variations in tonus. This tonic intramuscular pressure is found to be a prime factor in the venous return of blood to the heart. By means of Riml's experiment, particularly on a sympathectomized animal, it is shown that the tonic elasticity of the tissues is sufficient to squeeze a considerable part of the blood in the body out through the veins under a pressure corresponding to a column of blood of 10 or 12 cm.

The tonic intramuscular pressure is increased by a therapeutic dose of strychnine and by inhalation of carbon dioxide. It is decreased by overbreathing, and by illness, physical injuries and surgical operations.

The most powerful contraction of a muscle against resistance does not produce an internal pressure sufficient to stop the circulation of the blood through its vessels.

The tonic tissue pressure throughout the body combined with the negative pressure in the thorax, which is largely dependent upon the tonus of the respiratory muscles, determines the "effective venous pressure" that in health assures the venous return. Failure of the circulation in illness and after physical injuries and surgical operations is largely due to diminution of general body tonus and stagnation of the blood in the flaccid tissues (15).

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AIR MOVEMENT AS A STIMULUS TO THE SKIN, THE REFLEX EFFECTS UPON MUSCLE TONUS, AND INDIRECTLY UPON THE CIRCULATION OF THE BLOOD; ALSO THE EFFECTS OF THERAPEUTIC BATHS

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The effects of good and bad room ventilation upon comfort and health are now generally recognized as due to skin stimulation either by temperature and humidity or by air movement. The importance of air movement was emphasized to one of the authors by a recent experience in which under natural conditions in a large room (on a ship off the coast of Brazil) with a temperature of 80°F., and a high humidity he and another were quite comfortable so long as the air was kept in motion, but were almost nauseated as soon as they moved out of the range of the air from a ventilator.

At about the same time a paper by M. O. de Almeida (1) came to our attention in which he showed that not only in the frog, but also in rabbits, cats and dogs even a slight stimulation of the skin by contact with a solid body has a marked effect upon the tonus of the underlying muscles.

Some years ago Henderson (2) pointed out that the problem of ventilation is not merely that of external conditions applied to the body, but also that of the nature of the reactions within the body. It now occurred to us that perhaps these reactions consist in a reflex increase of general body tonus and associated functions induced by stimulation of the skin.

In accord with this idea we have learned, after our own investigations were complete, that Barcroft (3) has found that the fetus of a sheep delivered by Caesarean section in a warm bath has no tonus in its muscles until it is lifted out of the bath into the air; and that, if the cord is intact, the tonus disappears again when the fetus is replaced in the bath.

To this general problem we have applied the method of determining the degree of tonus by measurement of intramuscular pressure described in a preceding paper (4). At different times five healthy young men, naked to the waist, lay quietly in a specially designed room where the temperature was accurately regulated to 80°F., and the humidity to 80 per cent.

The tonic intramuscular pressure was determined in the left biceps.

The aim of the experiments was to compare the effect of the conditions 1, when the air in the room was absolutely still, and 2, when it was agitated by means of an electric fan. Arterial pressure was also measured under these

TABLE 1

Showing a marked increase of intramuscular pressure in the left biceps, but no increase of arterial pressure, in 3 healthy young men lying naked in a room at 80°F. and 80 per cent humidity, when the air was agitated by means of an electric fan

Intramuscular pressure in millimeters of water and arterial pressure in millimeters of mercury.

SUBJECT	FAN	ARTERIAL PRESSURE	INTRAMUSCULAR PRESSURE					AVERAGES
L. L.	Off	130/70	80	81	78	78		79
	On	130/77	137	137	136	135	134	136
D. L. H.	Off	140/76	64	63	62	60	64	63
	On	130/74	80	82	81	84	83	82
W. A. G.	Off	112/72	73	70	71	71		71
	On	108/72	94	93	89	96		93

TABLE 2

Showing the increase of intramuscular pressure in the left biceps induced by air movement in 2 healthy young men lying naked in a room at 80°F. and 80 per cent humidity

Arterial pressure and skin temperature were also altered by injection of adrenalin (1 cc. of 1:1000 solution) into the right biceps. Intramuscular pressures in millimeters of water; arterial pressure in millimeters of mercury.

MINUTES IN CONSTANT TEMPERATURE ROOM	NOTES	ARTERIAL PRESSURE		SKIN TEMPERATURE		INTRAMUSCULAR PRESSURE	
		A. W. O.	C. P. S.	A. W. O.	C. P. S.	A. W. O.	C. P. S.
30		130/82	118/68	95	94	71	63
35	Fan on						
45		130/83	122/68	91	91	89	78
50	Fan off						
65		132/82	120/68	94	93	74	65
70	Adrenalin injected						
105		152/87	148/78	90	88	79	69
120		150/85	145/76	90	89	68	67
125	Fan on						
135		142/82	140/74	90	90	85	75

two conditions; and the temperature of the skin of the feet was observed. In addition the effects of the injection of 1 cc. of a 1:1000 solution of adrenalin into the right biceps were determined on two of the subjects.

The results of these experiments are shown in tables 1 and 2.

From the data in table 1 it appears that, while there were individual differences in the degree of response to moving air, all three of the subjects developed a marked increase of muscle tonus and intramuscular pressure under this stimulus to the skin. On arterial pressure there was, on the contrary, little or no effect.

From the data in table 2 it appears that under these conditions the stimulus to the skin induced by moving air is mechanical rather than thermal. This is indicated by the fact that the cooling of the skin induced by adrenalin was accompanied by only a slight rise of intramuscular pressure, much less than the rise of that pressure under the influence of air movement with approximately the same change of skin temperature. It is interesting

TABLE 3

Showing the effects of therapeutic baths

Duration, 15 minutes. Temperature 92°F. Carbon dioxide content 700 cc. per liter.

SUBJECT	ARTERIAL PRESSURE MM. MERCURY		INTRAMUSCULAR PRESSURE MM. WATER		METABOLIC RATE, PER CENT OF NORMAL	
	Before bath	After bath	Before bath	After bath	Before bath	After bath
Dr. M.	120/68	116/66	75	78	+8.1	+8.7
H. D. H.	124/82	120/80	60	73		
T. C.	148/88	132/78	76	91		
L. A. G.	108/68	86/60	58	81	+6.1	+7.5
G. D. H.	114/66	106/64	75	109	+2.3	+12.7
C. P. S.					−4.3	+9.0
J. E.*	120/70	122/72	92	89		

* Normal water bath for 15 minutes at the same temperature.

to note also that, while the dose of adrenalin was sufficient to cause a considerable rise of arterial pressure, it had only a slight effect upon intramuscular pressure.

Influence of therapeutic baths on intramuscular pressure. If the general tonus of the body is influenced by skin stimulation, the therapeutic baths used in the treatment of cardiac disease should also induce a rise of intramuscular pressure. With this in mind, we availed ourselves of an opportunity, for which we are indebted to Dr. W. S. McClellan, to make the observations summarized in table 3 on the effects of baths in water supersaturated with carbon dioxide (700 cc. per liter) at the Saratoga Springs Sanitarium. The subjects were all normal, healthy men. It was found in control experiments that baths at the same temperature without carbon dioxide, of which one illustration is here reported, are without effect upon intramuscular pressure, or even induce a slight lowering. The stimulation

is due to the formation of innumerable minute bubbles upon the skin. This induced in all cases vasodilatation and a lowering of arterial pressure, but a rise of intramuscular pressure. The determinations of metabolism, although they suggest an increase coincident with the increase of tonus, lie within the limits of error of the method.

We are inclined to assign the physiological effects of carbonated baths to the stimulation of the skin.

But this stimulation is in some respects quite different from that of air movement. Under air movement the temperature and color of the skin were not appreciably altered, whereas the carbonated baths induced a very marked cutaneous hyperemia. The baths caused a considerable lowering of arterial pressure, while air movement had no such effect. Yet air movement had a greater effect upon intramuscular pressure than did the baths.

CONCLUSIONS

Among the principal effects of uncomfortable or comfortable room ventilation are the decrease or increase of the tonus of the musculature of the body induced by reflexes from the skin.

These facts and the relation of the circulation to muscle tonus, shown in the preceding paper, afford an explanation both of the stimulating effect of fresh air and of the syncope that sometimes occurs in crowded, ill ventilated halls. Fainting under such conditions, instead of being a vasomotor arterial failure, may be rather due to a failure of the venous return because of lowered muscle tonus.

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THE CARBOHYDRATE METABOLISM OF THE HEART DURING PANCREAS DIABETES

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The carbohydrate exchanges of normal hearts have been extensively studied through observations on the absorption of the glucose and lactic acid from the coronary blood (Himwich, Koskoff and Nahum, 1928; McGinty, 1931; McGinty and Miller, 1933; Evans, DeGraff, Kosaka, MacKenzie, Murphy, Vacek, Williams, Young, 1933; Himwich, Goldfarb and Nahum, 1934). More recently, the carbohydrate balance (glucose + lactic acid) of the diabetic heart has also been examined. In a preliminary note (Himwich, and Goldfarb, 1934) it was reported that the carbohydrate balance of the heart of depancreatized dogs examined *in situ* under artificial respiration was positive as in normal animals. Evans, Grande, Hsu, Lee and Mulder (1935) observed in the excised, perfused, diabetic heart a similar absorption of glucose and lactic acid. Since this preliminary report, additional data have been obtained on the respiratory metabolism of cardiac tissue as well as on its glycolytic powers the results of which are here presented.

METHOD. To determine the carbohydrate balance of the heart, 5 cc. samples of blood drawn practically simultaneously from the coronary sinus and the femoral artery were analyzed for glucose (Hagedorn and Jensen, 1923) following deproteinization (Somogyi, 1930) and lactic acid (Friedemann, Cotonio and Shaffer, 1924) after removal of reducing substances (Van Slyke, 1917). With these data, the total carbohydrate exchange of the hearts of dogs depancreatized 2 to 5 days was calculated. The animals were anesthetized with nembutal and the thorax was opened during the administration of artificial respiration.

Some observations of respiratory exchanges were made on the excised cardiac tissue of dogs, but in most instances, cats depancreatized 2 to 7 days supplied the cardiac tissues for these studies. The hearts were excised and sliced in thin sections of 0.2 mm. or less. The gaseous exchange of these sections was measured by the Warburg technique; glucose and lactic acid being used as substrates. The ability of cardiac tissue to glycolyze in a 2 per cent solution of Na_2HPO_4 with or without 0.2 per cent

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glucose was tested in 6 cats depancreatized 3 to 8 days and receiving no insulin, as well as in one depancreatized dog 7 days after the injections of insulin were stopped. The minced tissues were divided into 3 portions. To the first, iodoacetate was added immediately to prevent glycolysis; to the second, the drug was added in ½ hour; to the third, in 1 hour. Then all 3 portions were subjected to the Somogyi and Van Slyke precipitations and analyzed for lactic acid.

RESULTS. Twelve observations were made on 9 normal dogs, and 32 on 17 depancreatized animals. The glucose, lactic acid, and total carbohydrate balances of normal and diabetic hearts are presented in table 1.

TABLE 1

Glucose, lactic acid and total carbohydrate balances of the heart

	NORMAL			DEPANCREATIZED		
	+	0	−	+	0	−
Lactic acid	7	3	2	16	11	5
Glucose	9	1	2	19	7	76
Total carbohydrate	10	1	1	22	6	4

TABLE 2

R.Q. and O_2 consumption of the excised tissue of normal and diabetic animals with and without substrate

CONDITION	O_2 CONSUMPTION-100 MGM. TISSUE PER HOUR			R.Q.		
	Saline	Lactic acid	Glucose	Saline	Lactic acid	Glucose
Normal	108		124	0.83		0.90
Normal	69	121		0.81	1.00	
Diabetic	96		104	0.76		0.78
Diabetic	96	126		0.76	0.80	

Arteriovenous differences of 3 mgm. per cent or more for glucose and lactic acid, and of 6 mgm. per cent or more for total carbohydrate were considered significant. The number of occasions in which arteriovenous differences were positive, negative, or not significant is indicated in the respective columns (table 1).

The exchanges reveal that both the normal and diabetic hearts usually absorb lactic acid and glucose from the blood stream. The total carbohydrate balances were positive in 10 out of 12 observations of normal hearts, and 22 out of 32 of the diabetic organs.

Table 2 presents typical results of the respiratory metabolism of normal heart (4 experiments to determine the effect of the addition of glucose and 7 of lactic acid) and the diabetic organ (7 experiments each with glucose

and lactic acid). Each experiment represents the average of 4 observations.

In the normal tissues, the O_2 consumption rose in 2 out of 4 experiments with glucose and 6 out of 7 with lactic acid. With the diabetic tissues the O_2 intake increased only in 1 of 6 observations with glucose and in 4 of 7 with lactic acid. The R.Q. of diabetic cardiac tissue is not usually at the fat level and may be as high as 0.90.

The diabetic cardiac tissues are able to form lactic acid. The averages of the 6 experiments on cats are 90 mgm. per cent initially, 249 mgm. per cent at the half hour, and 389 mgm. per cent after one hour of glycolysis. The values obtained with the dog tissue for the same periods of glycolysis are 74 mgm. per cent, 370 mgm. per cent, and 400 mgm. per cent respectively.

Discussion. *Glycolysis.* The initial values for lactic acid of the cardiac tissues are somewhat high because they are not frozen before mincing, as in previous studies (Himwich, Goldfarb and Nahum, 1934). However, such a procedure is not necessary to show the ability of cardiac tissue to glycolyse. Glycolysis of diabetic cardiac tissue, moreover, is not peculiar to the feline species for the dog yielded similar results. The maximal values are not significantly different from those of Hines, Katz and Long (1925), nor from our own for the normal heart (unpublished data). Diabetic striated muscle, on the other hand, may exhibit a diminished glycolysis (Shorr, Loebel and Richardson, 1932).

Carbohydrate balance. The results reveal that the carbohydrate balance of the diabetic heart like that of the normal organ is positive. This absorption of carbohydrate is the underlying mechanism which makes possible the accumulation of glycogen in the diabetic heart (Cruickshank, 1913; Chambers, Kennard, Pollack and Dann, 1932). The greater storage of cardiac glycogen in the diabetic than in the normal cannot be imputed to diminished expenditure of energy, for it is probable that during diabetes the heart has a lowered efficiency. Hence, the high glycogen content must be due either to an absorption of carbohydrate greater than in the normal or to an impaired oxidation of carbohydrate. Evans, Grande, Hsu, Lee and Mulder (1935) observed a diminished absorption of carbohydrate by the diabetic heart. The greater glycogen content can therefore be explained best by a decreased oxidation of glucose, an explanation which is in accord with the impaired carbohydrate oxidation during diabetes.

This explanation of the paradoxical storage of cardiac glycogen in diabetes has, however, opened another question, for, if all the lactic acid and glucose absorbed were retained as glycogen, the heart might soon be so overloaded with that substance as to render it incapable of normal function. Since there is no evidence to show that glycogen leaves the heart as such or that in the fasting depancreatized animal, carbohydrate is transformed

to fat, and in the absence of any other method for the disposal of the glycogen, only the possibility of the oxidation of carbohydrate is left. The quotients reported in this study obtained by the Warburg technique furnish evidence that cardiac tissues of depancreatized animals can oxidize substances other than fat. In contrast, the renal tissues of these same animals yielded an R.Q. close to 0.7 (unpublished data). Bayliss, Muller, and Starling (1928) observed an R.Q. of approximately 0.8 in a heart-lung preparation of a diabetic dog. These high cardiac quotients are not in agreement with those of Cruickshank and Startup (1934) who also studied the perfused heart and noted quotients of 0.7 in a series of observations on dogs depancreatized 7 days or longer. It is possible that neither the perfusion method nor that of Warburg yields the same R. Q. as that of the heart in vivo. The determination of the R. Q. of the heart *in situ* was not successful, because the R. Q. may vary directly with the degree of ventilation, and for that reason excised tissue was resorted to.

The higher quotients reported by Bayliss, Muller and Starling (1928) and in the present paper could not be due to the oxidation of acetone bodies, as in striated muscle (Himwich, Goldfarb, Rakieten, Nahum, and Du Bois, 1934), since the chief source of the ketone acids, the liver, (Himwich, Goldfarb, Weller, 1931) was not able to supply these substances. Cruickshank, MacDonald, and Startup (1934) demonstrated that the mammalian heart was incapable of utilizing amino acids.

The oxidation of lactic acid may, however, be the limiting factor in the storage of cardiac glycogen, for in the present experiments cardiac tissues did not respond to glucose, but they did exhibit an increased O_2 consumption on the addition of lactic acid in 4 of 7 observations. Richardson, Shorr and Loebel (1930) found that diabetic voluntary muscle and kidney can oxidize lactic acid. Moreover, lactic acid is always available in the diabetic heart since it does not lose its ability to glycolyze. Evans and his co-workers (1931) also concluded that the diabetic heart may oxidize lactic acid, while McGinty (1931) believes that carbohydrate is usually oxidized in that form even in the normal animal.

To what extent lactic acid is oxidized *in vivo* is difficult to say. It would seem that the amount of lactic acid which is oxidized does not vary in the same manner as that of glucose, for during diabetes the oxidation of lactic acid is retained while that of glucose is lost. If no more lactic acid is oxidized by the diabetic heart than the normal, an increased proportion of the energy required for the function of that organ must arise from the oxidation of fat.

SUMMARY AND CONCLUSIONS

1. In 10 of 12 observations of normal dogs and in 22 of 32 of depancreatized animals, the carbohydrate balance (arteriovenous differences of glucose and lactic acid) of the heart *in situ* was positive.

2. These results can explain the paradoxical storage of cardiac glycogen in diabetes if a diminished ability to oxidize glucose is taken into consideration.

3. The R.Q. of diabetic cardiac tissue in a Warburg respirometer is usually above 0.7, indicating ability to oxidize non-fatty substances. The increased O_2 consumption in the presence of lactic acid indicates that its oxidation limits the storage of cardiac glycogen.

4. The ability of cardiac tissue to glycolyze is retained in pancreas diabetes.

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CONSTRICTION OF PIAL VESSELS IN THE UNANESTHETIZED CAT PRODUCED BY STIMULATION OF THE CERVICAL SYMPATHETIC CHAIN[1]

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The possibility of active constriction of cerebral arteries of sufficient intensity to produce cerebral anoxemia has been under discussion for many years. Recently, by direct observation through a window specially devised by Forbes (1) not to disturb normal intracranial pressure relationships, Forbes and Wolff (2) have demonstrated that stimulation of the cervical sympathetic chain is followed by ipsilateral constriction of the arterioles of the pia mater of anesthetized cats. The average reduction in diameter of such vessels upon stimulation was only 8.5 per cent, however, and it was suggested by the authors that the reflex response might be much greater in unanesthetized animals. The following experiments were devised to investigate the degree of vasoconstriction produced in normal unanesthetized cats by stimulation of the cervical sympathetic chain.

METHOD. Healthy young adult cats weighing 2 to 3 kgm. were used. Under nembutal anesthesia, the left cervical sympathetic chain was exposed aseptically and carefully freed from the vagus for a distance of several centimeters. The nerve was then gently encircled by a shielded silver wire electrode after the method of Bradford Cannon (3). Several days after the successful implantation of the electrode, the cat was again anesthetized with nembutal; under aseptic precautions, a trephine hole was bored in the left parietal region. The dura mater was left intact, and the open wound dressed.

Twenty-four hours after trephining the skull, when the cat was fully recovered from the anesthetic, a cubic centimeter of cerebrospinal fluid was drawn off by cisterna puncture, and the insensitive dura mater was removed with an electro-cautery. Externally the dura was covered with a tenacious sero-sanguinous exudate, but its inner surface was always smooth and free from adhesions, and the pia arachnoid appeared normal and

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delicate. The metal-rimmed window was quickly screwed into place and the air trapped beneath it was replaced by cerebrospinal fluid through the washout system, which was then tightly corked. The cat's body was snugly surrounded by wide bands of folded towels fixed to the longitudinal bars of the animal board; a cat so supported will lie perfectly still for periods of twenty or thirty minutes. Excellent painless immobilization of the cat's head was obtained with the head clamp devised by Forbes (1), although during its adjustment in the unanesthetized cat the cranial window was sometimes dislodged and the pial vessels traumatized. This difficulty could be avoided by encasing the cat's head, neck and thorax in a plaster cast with suitable windows for eyes, mouth, trephine hole and electrode wire while the cat was anesthetized on the preceding day, but when the head clamp was applied to the plaster shell, slight respiratory motion of the head persisted. The microscope and light were adjusted in the usual way, and the cervical and indifferent electrodes were connected with a Harvard induction coil.

The pial vessels were studied under low power and with the naked eye during sympathetic stimulation; by these methods any striking change in calibre could be easily observed. When immobilization of the head was complete, micrometer measurements of the arterial diameter were made under high power; at other times, even when respiratory head movements were present, small changes in caliber could be seen under high power ($\times$ 80) as the vessel moved to and fro in the field, but it was impossible to measure them accurately. The stimulus used always produced maximal dilatation of the ipsilateral pupil, with retraction of the nictitating membrane and widening of the palpebral fissure. The primary coil was connected to a single dry cell without external resistance, and the secondary coil was placed horizontally and its position varied from 11 to 7 cm.; in this range, the weaker currents were readily borne on the tongue. The period of stimulation varied from 15 to 50 seconds, but was usually 30 seconds.

Results. Three out of ten cats were considered satisfactory preparations at the time of the acute experiment, and all the observations reported were made on these three animals. The other seven cats were discarded for various technical reasons.

In cats 1 and 2, the head was held by the plaster cast method. Pial vessels were observed during eighteen stimulations of the cervical sympathetic chain. The position of the secondary coil was varied from 10 to 7. Under the high power lens at the end of stimulation, slight constriction of the vessel was uniformly noted. Under low power these changes in calibre were too small to be seen, although variations of 10 per cent or more are readily visible at this magnification.

In cat 3, perfect immobilization was obtained with the head clamp alone

and exact quantitative results were obtained. Adequate stimulation of the cervical sympathetic chain was followed by significant constriction of the pial artery in each of six trials. The amount of constriction ranged from 3 per cent to 8.3 per cent of the vessel's initial diameter; the average constriction was 6.4 per cent. The secondary coil during these six trials was set at 9 and 11 cm. from the primary coil. In three trials made with the secondary coil at 13 cm., there was no change in calibre of the pial artery, while the ipsilateral pupil dilated promptly; the stimulus in these three trials was therefore subminimal so far as the production of vasoconstrictor effects was concerned. In the six trials in which effective stimuli were used, the stimulus was applied for 30 seconds in five trials and for 60 seconds in one trial. Maximal constriction of the artery occurred usually 30 to 45 seconds after the beginning of stimulation, although once it was noted as early as 15 seconds after the stimulus started, and once the maximum of 8 per cent was reached two minutes after stimulation was begun, although 6 per cent constriction was observed at the end of the first 45 seconds. Maximal vasoconstriction was noted in 45 seconds in trial 5, although the stimulation was applied for a total of 60 seconds. It is noteworthy that in every trial maximal dilatation of the pupil was observed a few seconds after sympathetic stimulation was begun.

No changes in the calibre of capillaries or veins of the pia mater or in the color of the cortex were observed after sympathetic stimulation in any of the experimental animals.

Discussion. That constriction of pial arteries following sympathetic stimulation occurs in normal as well as in anesthetized cats is shown by these experiments; contrary to expectation, however, the order of magnitude of the reflex is essentially the same under the two conditions. Thus the average constriction which could be accurately measured was 6.7 per cent in unanesthetized cats while Forbes and Wolff (2) found an average constriction of 8.5 per cent in a much larger series of trials in animals anesthetized with amytal or ether. As in the anesthetized cats, the constrictor reflex of pial arterioles had a higher threshold to sympathetic stimulation than the pupillary dilator reflex. No single instance of striking arteriolar constriction was observed during 27 trials in normal cats using a weak interrupted faradic current as a stimulus.

SUMMARY

1. A method is described whereby it is possible to study changes in calibre of the pial vessels in unanesthetized cats.

2. Stimulation of the cervical sympathetic chain in unanesthetized cats was followed by slight constriction of the ipsilateral pial arterioles (average 6.7 per cent).

3. The constriction observed in unanesthetized cats was of the same order of magnitude and in no case greater than that reported in anesthetized cats.

I wish to express my thanks to Dr. Stanley Cobb for his helpful advice.

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ON VARIOUS PROPERTIES OF THROMBOPLASTIN (AQUEOUS TISSUE EXTRACTS)

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In accordance with the most commonly accepted theory of blood clotting, the process takes place in two steps:

I. Prothrombin + thromboplastin + calcium = thrombin
II. Fibrinogen + thrombin = fibrin

The mechanism of the first reaction, however, still remains a controversial question. Alexander Schmidt postulated a direct activation of prothrombin by zymoplastic substances, and this view, with the modification that calcium is equally essential, is still held by the majority of European investigators including Morawitz (1), Bordet (2), and Wöhlisch (3). Howell (4), however, maintains that thromboplastin merely neutralizes antiprothrombin, a substance normally occurring in the blood, which is combined with prothrombin, and which when removed allows the latter to react directly with calcium whereby thrombin results. Irrespective of which theory is correct, it is an experimental fact that tissue extract (thromboplastin) greatly accelerates the speed of clotting, and since it is fairly certain that the clotting time is proportional to the concentration of thrombin, it can be concluded that thromboplastin increases the rate of thrombin formation. Recently the writer made the interesting observation that when oxalated plasma was mixed with an active preparation of thromboplastin and recalcified with an optimal amount of calcium, a constant clotting time was obtained which could not be further shortened by additional thromboplastin. Since prothrombin is the only variable under the conditions of the experiment irrespective whether thrombin is formed by the liberation of prothrombin from antiprothrombin or by the direct interaction of thromboplastin with prothrombin, it follows that the clotting time must be dependent on the prothrombin concentration. On this basis the author (5) developed a new test for estimating prothrombin. By applying the test to a large number of human plasmas, a surprising constancy of the prothrombin concentration was found, and significantly this constituent was observed to be present in normal amounts in hemophilic

blood but often was greatly diminished in blood obtained from patients with various types of obstructive jaundice (6). By means of this new test it was further found that the prothrombin content of rabbit and dog blood was many fold greater than that of man. Such a finding has apparently not been noted heretofore, and obviously is of such importance that further investigation is warranted, in view of the possible far reaching consequences it may have both in the reinterpretation of many puzzling findings of previous investigators and in supplying new data on such subjects as coagulation from the standpoint of comparative physiology.

The first requisite in pursuing this subject further is a clear concept of the term thromboplastin. So much confusion concerning this substance exists at present that it is advisable to present a short historical summary preliminary to a new experimental approach. Alexander Schmidt discovered cellular products soluble in alcohol which greatly accelerated clotting and which he considered essential for the activation of prothrombin. He named these zymoplastic substances. Morawitz (7) likewise found aqueous extracts of various tissues exceedingly active in increasing the speed of coagulation. He emphasized the fact that these extracts could not clot fibrinogen by themselves, but only in the presence of prothrombin and calcium. He named his product thrombokinase. Fuld and Spiro (8) who obtained similar results introduced the term cytocyme which later Bordet (9) employed to designate his alcohol soluble platelet product. Howell suggested, and in his earlier work used the term thromboplastin. Nolf (10) whose complicated theory of clotting has been generally disregarded coined the term thrombocyme for the extract of platelets and leucocytes. Wooldridge (11) added to the complexity of the subject by postulating two types of fibrinogen, A and B, which interacted to form a clot. He believed that tissue extract contained tissue fibrinogen equivalent to his fibrinogen A. He recognized the phospho-lipoid character of tissue extract and postulated that the active component was a lecithin-protein. It must be remembered that in Wooldridge's time no sharp distinction between the lipoids was recognized and lecithin partook of the nature of a generic term for phospho-lipoids. In recent years Mills (12) has revived the Wooldridge theory in the form that there are two distinct clotting processes, one brought about by thrombin, the product of the union of free cephalin with prothrombin, the other by tissue fibrinogen, a cephalin-protein compound.

With the introduction of these various terms, the subject became increasingly complicated. Motivated no doubt by the desire to clarify the clotting problem through the isolation of the active constituent in platelets and other tissue extracts, a number of investigators studied the chemical nature of various zymoplastic preparations. These investigations which were started almost simultaneously around 1910 by Zak,

Bordet, and Howell, led to the important conclusion that the active principle is a phospho-lipoid, which Howell (13) and his student McLean (14) identified as cephalin. It is not surprising that these outstanding contributions caused many subsequent workers to accept tacitly that free cephalin is identical with thromboplastin. Yet Howell explicitly stated, "We must conclude that in the brain and thymus and no doubt other tissues generally the thromboplastin phosphatid material is combined with a tissue protein having a low temperature of heat coagulation" (13). It seems very probable that in this statement is the key to a better understanding of the true nature of thromboplastin. Even Wooldridge, it will be recalled, considered tissue fibrinogen to be a lecithin-protein compound. It is an accepted rule that in the isolation of an active principle, whether it be a hormone, a vitamin, or an enzyme, the product should show a greater potency than the mother substance. Yet, an aqueous extract of brain is far more active than pure cephalin or any ether or petroleum ether extract, a finding which makes it appear doubtful whether free cephalin is the true stimulator of thrombin formation. The results obtained in the present investigation suggest that thromboplastin is a complex in which cephalin is an essential constituent.

EXPERIMENTAL. *New prothrombin test.* The method which the writer developed for the estimation of prothrombin has been found to be especially well suited for the determination of thromboplastic activity. As already stated, the test consists in mixing oxalated plasma with an active preparation of thromboplastin, and noting the clotting time after adding an optimal quantity of calcium. The actual procedure was as follows:

With special precaution to avoid trauma, 9 cc. of blood were rapidly withdrawn into a syringe containing 1 cc. of 0.1 M sodium oxalate. In the case of the rabbit, cat, and guinea pig, blood was taken directly from the heart. The necessity of preventing the formation of thrombin must be stressed; speed in taking the blood, and promptness and thoroughness in mixing it with oxalate were found to be of utmost importance. As a further precaution chilled syringes and tubes were used. The blood was centrifuged soon after it was taken. In a small test tube (13 x 100 mm.) 0.1 cc. of plasma was mixed with 0.1 cc. of thromboplastin emulsion, and 0.1 cc. of 0.025 M calcium chloride added. The tube was immediately placed in a water bath maintained at 37°C., and the exact time required for the formation of a solid clot noted.

In a previous study evidence was presented to show that the clotting time under the conditions of the experiment as outlined is a specific measure of the prothrombin concentration. It is unnecessary at this time to discuss the validity of this conclusion, but it should be stated that for the blood of any particular species, the clotting factor which responds to thromboplastin, whether it be prothrombin or prothrombin-antiprothrombin, is

remarkably constant. In a series of over 150 specimens of human blood, using a preparation of thromboplastin of constant activity, the test showed a mean variation of only about 1 second in the clotting time. Human plasma therefore is highly satisfactory for testing the activity of thromboplastin. In the present study, however, rabbit blood was mainly used since it was more convenient to obtain and appears to be equally satisfactory.

Theoretically, freshly drawn blood could be used instead of oxalated plasma, for on adding 0.1 cc. of thromboplastin to 1 cc. of human blood and determining the clotting time according to the technique of the Lee-White method, coagulation occurs in 25 to 30 seconds, whereas without thromboplastin the clotting time is 4 to 8 minutes. Practically, whole blood is unsatisfactory, for it requires taking blood for each determination and it does not permit the exact timing of the clot formation as is possible with a transparent medium such as plasma. Howell's method of testing thromboplastin on dog plasma made incoagulable by peptone injection can also be used as illustrated by the following experiment:

Blood was taken 7 minutes after injecting 10 cc. of a 20 per cent solution of peptone into a dog weighing 7 kgm. On mixing 0.1 cc. of this unoxalated plasma with 0.1 cc. of thromboplastin, a clot was obtained in 7.5 seconds. As a control 0.1 cc. of oxalated plasma obtained before the injection of peptone + 0.1 cc. thromboplastin + 0.1 cc. 0.025 M calcium chloride, gave a clot in 8 seconds.

The experiment illustrates that unoxalated plasma obtained after injecting peptone acts similar to oxalated plasma and that such plasma could be used to determine thromboplastic activity. The method does not offer any advantages over the simple procedure using oxalated plasma and is limited in its application.

Preparation of thromboplastin. It was found that an aqueous emulsion of rabbit brain possesses an exceedingly high thromboplastic activity, and that the lungs and thymus yielded an equally potent extract. Brain, however, is the most satisfactory, since it is easily freed of blood, can readily be emulsified, and moreover can without appreciable loss of activity be dried and in the anhydrous state be preserved with no appreciable loss of potency for months. The preparation was as follows:

The organ or tissue of a freshly killed rabbit was freed as much as possible of all blood and blood vessels, then washed, weighed, and thoroughly macerated in a mortar. Sufficient 0.85 per cent sodium chloride solution was added to make a 10 per cent extract. The mixture was heated to 60°C. for 15 minutes to inactivate any traces of thrombin and prothrombin arising from blood remaining in the tissue. The solid material was removed by centrifuging and the opalescent liquid used directly. To prepare the dry material, a rabbit brain was freed of blood by removing the pia

mater, then weighing and macerating as before. The pulp was uniformly spread on several layers of gauze, and dried at 37°C. For convenience, the impregnated gauze was cut into pieces representing 1 gram of fresh material. To prepare the thromboplastic emulsion, one piece was leached with 10 cc. of normal saline solution, heated to 54°C. for 10 minutes, and centrifuged. The preparation thus obtained showed the same activity as that made from fresh tissues. With these methods, a preparation can be obtained which when tested by the new prothrombin method causes human plasma to clot in 16 to 19 seconds, and rabbit and dog plasma in 7 to 9 seconds. In the earlier part of this work a less active preparation was obtained, and the present evidence indicates that there may be a

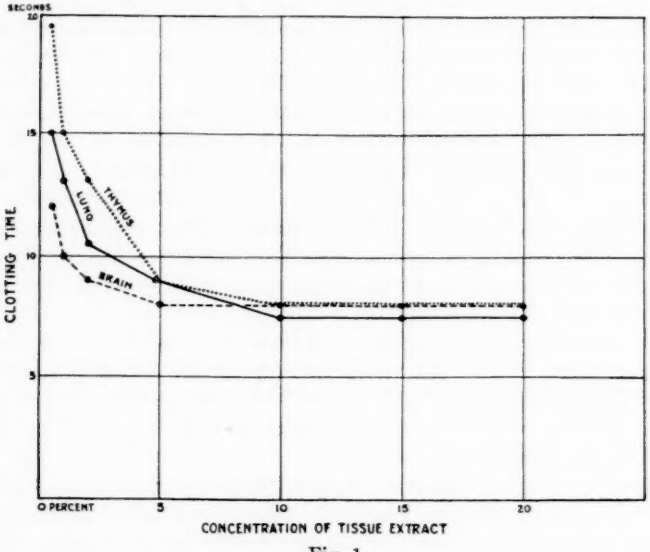

Fig. 1

seasonal variation in thromboplastic activity, but this requires further study before any definite conclusions can be drawn.

PROPERTIES OF THROMBOPLASTIN. *1. Has fixed degree of activity.* On testing the thromboplastic activity of increasing concentrations of a tissue extract by the new prothrombin test, it was found that the clotting time reached a minimum which could not be appreciably shortened by additional thromboplastin as shown by the results in figure 1.

This minimum clotting time can be considered the true measure of the activity of any preparation of tissue extract, and it will be observed that the minimum clotting time is essentially the same whether the extract is made from brain, lung, or thymus, thus suggesting that the active sub-

stance in these three tissues is probably the same. It is interesting to recall that Howell in 1912 wrote: "There is every reason to believe that the thromboplastic substance is essentially the same in all tissues and acts in all cases in the same way" (13).

But interesting variations in activity can occur even in different animals of the same species. The fluctuation in activity observed in rabbits, which has already been mentioned, is not great enough to alter or affect the conclusions drawn from the results presented in this paper, but nevertheless presents a problem which will receive further study. A comparison of the activity of tissue extracts from animals of different species is taken up later.

As thromboplastin deteriorates its activity decreases, i.e., the minimum clotting time that it produces, under standardized conditions of the author's test, becomes longer; and significantly, using larger amounts or greater concentrations of the less active preparation will not shorten the clotting time. The activity of dehydrated tissue extract decreases slowly, but it can be rapidly diminished by extraction with a lipoid solvent or by heat.

The degree of activity must not be confused with thromboplastin content. By examining figure 1, it will be seen that the clotting time is only influenced by the concentration of thromboplastin if it is below the critical level. It should also be noted that the content of thromboplastin in the lung is apparently not very different from that of the brain, but in agreement with the observations of others the lungs occasionally show a somewhat greater activity than do other tissues.

It is interesting that Mellanby (15) in 1909 noted that thrombokinase beyond a certain limit did not further decrease the coagulation time, and Wakesman (16) working with cephalin prepared according to Howell's method noted that after a certain concentration of cephalin in water was reached, the clotting time could not be further shortened by greater concentrations of cephalin. The possibility that this property could be used as a measure of thromboplastic activity as well as a means for estimating prothrombin, seems to have been overlooked by these authors as well as by subsequent workers.

2. Activates prothrombin; cannot clot fibrinogen in the absence of prothrombin. To determine whether the acceleration in clotting time produced by thromboplastin is due to an increased formation of thrombin, or to a direct clotting action of thromboplastin, as Mills postulates, its action was tested on purified fibrinogen and on prothrombin-free plasma.

Fibrinogen was prepared as follows: 5 cc. of oxalated human plasma were diluted with 10 cc. of 0.85 per cent sodium chloride, and mixed with 5 cc. of saturated ammonium sulfate solution. The precipitated fibrinogen was removed by centrifuging, redissolved in 15 cc. of normal saline solution, and reprecipitated with 5 cc. of saturated ammonium sulfate solution. The

above process was repeated and the fibrinogen again removed and tightly packed by rapid centrifuging, and the ammonium sulfate solution poured off, and the last traces removed by drying the tube with filter paper. The fibrinogen was redissolved in 5 cc. of normal saline solution, and after dialyzing for 1 hour against a normal saline solution was ready for use. By employing human plasma, which the writer has found to contain much less prothrombin than rabbit or dog plasma, it is relatively easy to obtain a product which does not contain demonstrable amounts of prothrombin. No clot was obtained when this fibrinogen solution was mixed with thromboplastin as seen in table 1.

The finding that tissue extract fails to clot fibrinogen even in the presence of calcium agrees with the observations of Smith, Warner, and Brinkhous (17) who demonstrated that lung extract that had been carefully freed of blood did not clot purified fibrinogen. Their procedure for washing out blood by bleeding and simultaneously injecting saline ante-mortem has been found highly satisfactory especially for larger animals.

TABLE 1

Inability of tissue extract to coagulate purified fibrinogen

Fibrinogen solution, cc.	0.1	0.1	0.2	0.2	0.3	0.3
Thromboplastin emulsion, cc.	0.1	0.1	0.1	0.1	0.1	0.1
Calcium chloride 0.025 M., cc.	0.0	0.1	0.0	0.1	0.0	0.1
Clot	0	0	0	0	0	0

Fibrinogen, 0.1 cc. + freshly prepared serum, 0.1 cc., gave solid clot in 10 seconds.

To obtain further evidence for or against the view that tissue extract functions solely as the activator of prothrombin, its action was tested on plasma deprived of prothrombin. Interestingly, aluminum hydroxide cream removes or inactivates prothrombin without apparently causing any other significant change in the plasma as the writer has recently reported (18). Aluminum hydroxide is an exceptionally mild reagent, and is neutral in its reaction. It has no demonstrable effect on fibrinogen, for plasma treated with this reagent will readily clot when thrombin is added, and the amount of fibrin obtained is the same as in normal plasma. Plasma treated with aluminum hydroxide therefore is especially suitable to test the action of thromboplastin, since the fibrinogen is still in its natural medium and less apt to be altered than is the purified product.

The procedure for prothrombin-free plasma was as follows: 1 cc. of oxalated plasma was mixed with 0.1 cc. of a thick aluminum hydroxide cream, and incubated for 15 minutes at 37°C. On centrifuging at a high speed, a perfectly clear plasma was obtained which for convenience and brevity has been designated by the writer as alumina plasma.

From the results recorded in table 2, it can be seen that thromboplastin of a high degree of activity fails to clot plasma deprived of prothrombin. The failure of tissue extract to clot either fibrinogen or prothrombin-free plasma demonstrates that it does not possess in itself the power to bring about coagulation, thus confirming the first postulate of Morawitz (7). The results definitely indicate that the true function of tissue extract or thromboplastin is the activation of prothrombin. This conclusion is con-

TABLE 2

Ineffectiveness of tissue extract (thromboplastin) to bring about clotting in plasma from which prothrombin has been removed with aluminum hydroxide

	cc.		cc.
Alumina plasma*†	0.1	Alumina plasma	0.1
Calcium chloride 0.025 M	0.1	Thromboplastin‡	0.1
		Calcium chloride 0.025 M	0.1
No clot in 24 hours		No clot in 24 hours	
Oxalated plasma	0.1	Oxalated plasma	0.1
Calcium chloride 0.025 M	0.1	Thromboplastin	0.1
		Calcium chloride 0.025 M	0.1
Solid clot in 105 seconds		Solid clot in 16 seconds	
Oxalated plasma	0.1	Oxalated plasma	0.1
Alumina plasma	0.1	Alumina plasma	0.1
Calcium chloride 0.025 M	0.2	Thromboplastin	0.1
		Calcium chloride 0.025 M	0.2
Solid clot in 90 seconds		Solid clot in 22 seconds	
Alumina plasma	0.1	Oxalated plasma	0.1
Fresh serum	0.1	Fresh serum	0.1
Solid clot in 15 seconds		Solid clot in 15 seconds	

* Plasma treated with aluminum hydroxide to remove prothrombin.
† Human plasma employed in all experiments.
‡ Thromboplastin prepared from rabbit brain.

trary to Mills who maintains that tissue fibrinogen can clot fibrinogen independent of thrombin. Mills' tissue fibrinogen is obtained by the extraction of lung tissue with a physiological saline solution. In view of the difficulty with which blood is removed from the lungs as Smith and his coworkers (17) have demonstrated, it is fairly probable that tissue fibrinogen consists of thromboplastin containing traces of prothrombin. A more extensive study of Mills' tissue fibrinogen will, however, be presented in a subsequent study.

3. Thromboplastic activity diminished by extraction with lipoid solvents. On extracting carefully dried lung or brain with any of the common immiscible solvents, the thromboplastic activity is greatly diminished, and the extract after the removal of the solvent shows a relatively small thromboplastic action as shown in table 3.

It will be noted that the effect of different solvents is not the same. A dry preparation of rabbit lung or brain when extracted with ether loses much of its activity, nevertheless the extract after the removal of the ether has very little thromboplastic potency. With a low boiling petroleum (20 to 40°C.), the extract likewise is only slightly active, whereas with a higher boiling fraction (40 to 60°C.) the extract is somewhat more active. Yet, with either fraction the extracted residue retains approximately the

TABLE 3

The effect on thromboplastic activity produced by extracting dehydrated tissue extract (rabbit brain) with lipoid solvents

SOLVENT	CLOTTING TIME		
	Extract	Residue	Combined extract and residue
	seconds	*seconds*	*seconds*
Ethyl ether	85–95	35–40	35–40
Petroleum ether, B.p. 20–40°C	50–60	35–40	35–40
Petroleum ether, B.p. 40–60°C	35–40	30–40	30–40
Carbon tetrachloride	55–65	55–60	50–60
Acetone	50–65	11–13	10–14

The equivalent of 2 grams of fresh brain was extracted for 2 hours in a continuous extractor. The residue was leached with 10 cc. of normal saline solution, and the extract after the removal of the solvent was mixed with 3 cc. of saline solution. The thromboplastic activity was tested on rabbit plasma which without added tissue extract clotted in 85 to 105 seconds.

same thromboplastic activity. Carbon tetrachloride effectively diminished the potency of the extracted residue but the extract has little activity. Acetone interestingly seems to extract a small amount of thromboplastic material but even after two hours of vigorous extraction the residue still retains a relatively high degree of activity.

From the intensive studies of Howell (13), McLean (14), and Gratia and Levene (19) the substance responsible for the thromboplastic activity of the extract has been identified as cephalin. The important question, however, remains whether this extracted lipoid exists as such in the tissue extract or whether it is liberated or split off from a larger molecule in the course of the extraction. If the active lipoid exists uncombined, its removal from the dry preparation should readily be effected by ether or

petroleum ether, and the activity should reappear quantitatively in the extract, which obviously does not occur. It seems more proabable that thromboplastin is a conjugated cephalin which is disrupted by lipoid solvents. The fact that the original activity is not restored by mixing the emulsified extract with the residue indicates that thromboplastin is not readily resynthesized, and further suggests that it is probably a definite substance rather than a loosely adhering complex of cephalin with an inert colloidal aggregate.

4. Thromboplastic activity diminished by heat, but not totally destroyed. When an aqueous emulsion of tissue extract is heated, no significant alteration in activity is observed until the temperature is elevated above 60°C., when its potency begins to be definitely diminished. But even boiling it for an hour does not inactivate it. Curiously, the aqueous extract of brain

TABLE 4

The effect of heating on the thromboplastic activity of tissue extract

TEMPERATURE	CLOTTING TIME	
	Extract of rabbit brain	Extract of rabbit lung
°C.	*seconds*	*seconds*
54	8	7.5
60	8	8
75	25	12
90	30	15
100	37*	13*
	50†	20†

The clotting time was tested on rabbit plasma. The tissue extract was heated for 15 minutes at the temperature specified.

* Heated for 10 minutes.

† Heated for 60 minutes.

is much more affected by heat than is a similar preparation of lung. In fact, the latter remains relatively active after boiling. No satisfactory explanation for this finding can be given. One must question, however, certain commonly held views which are perhaps best expressed by Ferguson (20): "The presence of the protein moiety renders tissue coagulant labile to heat (over 60°C.). After boiling it has the properties of cephalin alone which is heat stable." According to this statement one would expect that the extract of brain, which contains considerably more cephalin than does lung, should show the greater activity after heating, but diametrically the opposite is observed experimentally. Furthermore, the activity of the lung preparation even after having been heated to boiling for an hour, still has a much greater activity than any ether or petroleum ether extract. These findings suggest that the activity remaining in tissue extract after

heating is still due to the active principle, thromboplastin, which apparently is not as labile to heat as is generally assumed. Interestingly, tissue extract in the dehydrated form can be heated to 110°C. for an hour without any appreciable loss in potency.

5. Thromboplastin appears to possess to a limited degree species specificity. Lee and Vincent (21) reported that "thrombin required a longer time for its formation in human blood than in rabbit blood." No subsequent attempt was made to evaluate or explain this finding until recently when the writer (5) discovered that the acceleration of clotting by thromboplastin was much greater for rabbit and dog blood than for human blood; and concluded that the difference in response to thromboplastin could be explained by the assumption that the concentration of prothrombin was much greater in the blood of the dog and rabbit than in that of man. While further study supported the essential correctness of this conclusion, the present investigation disclosed the possibility of another factor, species specificity, as probably an important property of thromboplastin. The recognition of this possibility is antedated by work reported over 30 years ago which apparently has been completely ignored or forgotten. Loeb (22) noted the specificity of tissue extract of the lobster for its own blood, and he obtained evidence of such a specificity even among the vertebrates. Hewlett (23) observed that an extract made from goose liver did not accelerate the clotting of dog blood, but the extract of dog liver was active in clotting goose blood. Very recently Mendeléeff (24) in studying the accelerating power of embryonic guinea pig tissue extracts on blood clotting, noted that they were very effective for guinea pig plasma, less for rabbit plasma, and they failed to clot chicken plasma. Interestingly, Bordet, Renaux, and Bordet (25) could demonstrate a serological specificity of the cytocyme of various species, but failed to note such a difference in the thromboplastic activity.

The thromboplastic activity of various tissue extracts was determined by the author's new prothrombin test. A 10 per cent aqueous extract of the brain and lungs of various types of animals was used. The technique of Smith, Warner, and Brinkhous (17) was used to obtain lung tissue free of blood.

On examining the results presented in table 5, one notes immediately that the thromboplastin in the lung and brain in any one species has approximately the same degree of activity, thus further substantiating the conclusion that in any particular animal the thromboplastin in all tissues is probably identical. One can therefore assume that the activity of the lung and brain extracts is a measure of the activity of the thromboplastin for the particular animal studied. A distinct difference, however, exists in the activity of the thromboplastin in the four types of animals studied. The preparations made from rabbit and cat tissues appear the most active,

those of the dog somewhat less potent, while those of the guinea pig show little activity except for guinea pig blood itself. On the other hand, the response of the blood to thromboplastin is in a somewhat different order. Rabbit and dog blood show the shortest clotting time, cat blood a slight but definitely longer coagulation rate, while the blood of man and of the guinea pig has a much more protracted clotting time. There is a tendency for the tissue extract of any one species to show its greatest activity when tested on its own blood, but of the four animals studied, only the guinea pig strikingly demonstrates this property. The extract prepared from the lungs and brains of this animal exhibits little thromboplastic activity except when tested with guinea pig blood, which it causes to clot in 15 to 17 seconds. Curiously, guinea pig blood responds, though only moderately, to the thromboplastin of other animals.

TABLE 5

A comparison of the thromboplastic activity of tissue extracts (brain and lung) from different common laboratory animals when tested on the plasma of these and of man

SOURCE OF THROMBOPLASTIN		CLOTTING TIME				
		Rabbit plasma	Dog plasma	Cat plasma	Guinea pig plasma	Human plasma
		seconds	*seconds*	*seconds*	*seconds*	*seconds*
Rabbit	Brain	7–9	7–9	11–13	30–35	16–19
	Lung	7–8	8–10	9–11	27–30	16–19
Dog	Brain	10–11	6½–8	12–13	30–35	25–30
	Lung	8–11	6½–8	11–13	30–35	25–30
Cat	Brain	7–9	7–8	8–9	25–30	18–20
	Lung	7–8	6½–8	7–8	22–25	17–19
Guinea pig	Brain	40–45	40–55	60–65	15–17	60–85
	Lung	40–45	35–40	55–65	16–18	60–80

Human blood responds to tissue extracts of various animals but to a less extent than does cat, rabbit, or dog blood. Extracts of human brain and lung were not obtainable, therefore the effect of these on human as well as on other bloods was not determined. Significantly, however, human saliva showed a greater thromboplastic effect on dog and rabbit plasma than on human plasma. By mixing 0.1 cc. of plasma with 0.1 cc. of clear saliva, and promptly recalcifying with 0.1 cc. of 0.025 M calcium chloride solution, both dog and rabbit plasma clotted in 27 to 30 seconds, while the human plasma required 45 to 50 seconds.

The explanation for the present results is difficult until much more information is available. A few deductions can, however, be made, and a few possibilities pointed out. The view, that the greater activity of the aqueous tissue extract as compared to free cephalin is due to the fact that in the

extract cephalin is bound with a molecule which increases its solubility and therefore its activity, does not appear to be adequate. As already pointed out, an aqueous extract of guinea pig brain or lung has a relatively weak thromboplastic action on cat, rabbit, and human plasma, yet it is relatively potent for its own blood. Obviously, the solubility of cephalin cannot be the basic factor in this instance. Furthermore, guinea pig brain presumably contains as much cephalin as does the brain of the other animals used in this study; nevertheless, its thromboplastic action is markedly different, which makes it difficult to look upon cephalin as being the sole constituent responsible for the thromboplastic activity of tissue extract.

A priori, the results with guinea pig extracts suggest that thromboplastin possesses species specificity. If this be true, it must be admitted that the specificity is far from being absolute, and in the other animals studied there is at best only a suggestion of this property. But the possibility exists that a greater difference will be found when animals less closely related are studied. It should be recalled that Hewlett found the extract of goose liver had no effect on the clotting of dog blood. Assuredly the question of species specificity cannot summarily be dismissed. But this leads to another complication, namely, if thromboplastin is species specific, the prothrombin with which it reacts may possibly also have a corresponding specificity. Yet, the end product, thrombin, is assuredly not species specific, since it has been found to react with fibrinogen of many different sources.

Undoubtedly an important factor influencing the response of the plasma's clotting time to thromboplastin is the concentration of prothrombin. The slower clotting rate of human plasma as compared to that of the cat, dog, or rabbit, when treated with excess thromboplastin is best explained by the assumption that the blood of man contains less prothrombin. That it is not accounted for by species specificity is fairly convincingly demonstrated by the fact that the thromboplastin of human saliva is more effective in accelerating the clotting of dog and rabbit plasma than of human plasma.

Comment. The results obtained in the present investigation support the theory that tissue extract contains a substance which accelerates clotting by increasing the rate of thrombin formation. This agent, known under various names of which thromboplastin seems the most preferred, cannot by itself convert fibrinogen to fibrin. In plasma devoid of prothrombin, it is wholly inactive, thus demonstrating that its essential function is the activation of prothrombin. How this conversion of prothrombin to thrombin is brought about by thromboplastin remains unanswered.

Thromboplastin does not appear to be identical with free cephalin, since it greatly exceeds the latter in activity. It is more probable that it con-

sists of a complex of which this phospho-lipoid is an essential constituent. No evidence can be found that free cephalin functions in the normal physiological clotting of blood; nor does it appear certain that cephalin is the only essential constituent of the thromboplastin complex. The greater thromboplastic activity of an aqueous extract of lung has been generally recognized, and in the present study evidence is presented suggesting that the content of thromboplastin in this tissue and in the brain is of the same order of magnitude. Yet, it is well known that the brain is exceedingly rich in cephalin, whereas lung is relatively poor in this lipoid as Sammartino (26) has determined. The fact that the thromboplastic activity of these two tissues is nevertheless practically the same, suggests that it is not the amount of cephalin, but rather the fraction bound with an equally essential unknown component, which constitutes thromboplastin. The nature of the non-lipoid component is entirely unknown, but the fact that heating does not completely inactivate it, makes one question whether one is justified to consider it a protein. If thromboplastin possesses some degree of species specificity, as the results obtained with guinea pig tissue extracts suggest, it is interesting to speculate whether this property is dependent upon the lipoid component or upon an unknown fraction of the molecule.

SUMMARY

1. The activity of thromboplastin was quantitatively studied by determining the clotting time resulting when a fixed quantity of oxalated plasma was mixed with an aqueous extract of the tissue studied, and recalcified with an optimal amount of calcium chloride.

2. Extracts of rabbit brain, lungs, and thymus were prepared by freeing these tissues as thoroughly as possible of blood, macerating, mixing with physiological saline solution, heating to 60°C., and then centrifuging.

3. The following properties of tissue extracts were observed:

A. On testing the thromboplastic activity of increasing concentrations of tissue extract a minimum clotting time was reached, which could not be appreciably shortened by additional thromboplastin. No appreciable difference in activity between the extracts of brain, lung, or thymus of the rabbit was found. Likewise, the concentration of thromboplastin in these tissues appeared to be of the same order of magnitude.

B. The tissue extracts failed to clot purified fibrinogen or plasma from which the prothrombin had been removed by aluminum hydroxide cream.

C. On extracting dehydrated extract of rabbit brain with lipoid solvents the thromboplastic potency of the extracted material was markedly diminished, but the extract showed only a slight activity.

D. On heating aqueous tissue extracts, the thromboplastic activity decreased with increasing temperature. The extract of brain was found more

labile than that of lung. Even after heating the latter at 100°C. for 1 hour, it still remained fairly active.

E. A distinct difference was found in the thromboplastic activity of tissue extracts (lung and brain) prepared from various common laboratory animals, as well as a difference in the response of the blood of these animals to thromboplastin. This was most pronounced in the guinea pig, the tissue extract of which showed only slight thromboplastic potency except for its own blood, and the blood only moderate response to tissue extracts of other animals. These findings suggest that thromboplastin may be to some extent species specific. In human blood, the less active response to thromboplastin appears to be due to a smaller concentration of prothrombin than is present in the plasma of the cat, dog, and rabbit.

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THE ADRENAL CORTEX AND ENDOGENOUS CARBOHYDRATE FORMATION

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In a previous communication (Evans, 1934) it was reported that albino rats previously 24-hour fasted, placed for a further 24-hour fast in a well-ventilated chamber at ½ atmosphere contain at the end of that time 34 per cent more glycogen than control 48-hour fasted rats, the increase occurring chiefly but not exclusively in the liver. The phenomenon was found not to occur in bilaterally adrenalectomized animals.

The present paper undertakes to present certain findings which corroborate the previous ones and which bear upon questions arising from them, namely: Does the extra carbohydrate take origin from protein or fat or from both? Is the cortex or medulla, or is the whole adrenal gland essential for the phenomenon? If, as already suggested, the adrenals are concerned in the endogenous formation of carbohydrate, can this be demonstrated in the phlorhidzinized animal?

METHODS. For the collection of urine of fasting rats, metabolism cages consisting of a cylindrical wire cage provided with drinking water and sitting in a large funnel were used, one rat being placed in each cage. The feces, caught on a perforated plate part way down the funnel were removed before washing the plate and funnel. The urine was collected under oil and in the case of phlorhidzinized rats, sodium fluoride was used for the preservation of sugar. The urine and added washings were diluted to 100 cc. in a volumetric flask. From this, aliquots were taken for the determination of sugar, nitrogen and ketones. The fraction used for nitrogen determination was shaken with ⅕ its volume each of 10 per cent sodium tungstate and ⅔ N. sulphuric acid, and filtered to remove any protein. The filtrate was then further diluted to such a volume that 1 cc. contained approximately 0.5 to 1.0 mgm. of nitrogen. Several methods of Kjeldahl digestion were tried and although each gave good recoveries of nitrogen from weighed quantities of urea, none of them gave satisfactorily uniform values for the diluted rat urine. After further trials the following procedure was decided upon: 1 cc. of diluted rat urine containing approximately 0.5 to 1.0 mgm. of nitrogen, 1 cc. of concentrated sulphuric acid

and a pinch of an equal mixture of selenium powder and washed talc were placed in a 100 cc. Pyrex Kjeldahl flask and boiled over a moderate flame for 15 minutes. After partial cooling, 10 drops of Merck's superoxol were added and heating resumed for 3 minutes. After cooling, the digest was estimated for ammonia by distillation in a Pregl apparatus. The average disagreement in twelve successive duplicate analyses on twelve different urines was 1.8 per cent.

The urinary sugar was determined by the Shaffer-Hartman macro method, the urinary ketones by the method of van Slyke.

In the experiments in which urine collections were made during exposure to ½ atmosphere the whole metabolism cage was placed inside the chamber previously described (Evans, 1934).

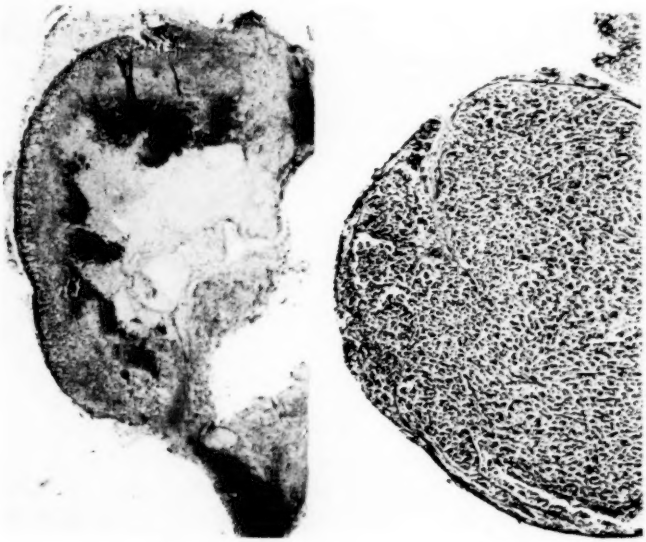
Phloridzination was accomplished by the daily subcutaneous injection of 50 mgm. of phlorhidzin suspended in 1 cc. of olive oil, such dose being divided and injected in two sites. The animals were fasted and the urinary collection begun from the time of the first injection.

The so-called demedullation of the adrenals was performed as follows: Each adrenal was brought into the opening of the lumbar incision and held there by a small curved forceps shielded with soft rubber and so constructed that it could not close completely. The forceps surrounded the adrenal artery and vein and held the adrenal in place only by virtue of the fact that the adrenal could not slip back through the limited space between the forcep points. One half of the adrenal was cut away and by careful squeezing of the remaining half the residual medulla with a generous coating of cortex could be removed. All that was left of each adrenal was one half of the capsule with a thin lining layer of cortical cells. It is the reparative process following the operation that provides what, for want of a better term, has been called a demedullated adrenal. The reparative process has been followed by removal and serial section (i.e., a section every 500 micra) of the operated glands. Three days post-operatively the sections revealed a hemorrhagic coagulum with at its periphery a few surviving cortical cells (fig. 1a). In 10 to 18 days, time the glands consisted of newly formed cortical cells in columnar arrangement, the central portion showing either a scar or an area of looser connective tissue containing granules of hemosiderin. No chromaffin tissue was found in any of the sections and the glands of all test animals were so sectioned.

Hypophysectomy was performed by a combination of the techniques kindly demonstrated by Dr. Hans Selye of McGill University and Dr. Robert Phillips of the New Haven General Hospital.

For the determination of oxygen consumption and respiratory quotient the Haldane open circuit method was used. The periods of observation varied between 3 and 4 hours; the apparatus was tested frequently to provide against possible leaks. The temperature of the circulating air was

held at approximately 26°C. In the case of animals exposed to low oxygen tension a mixture of 10.5 per cent oxygen in nitrogen was used. In all cases the animals previously 24-hour fasted were placed in the chamber in a stream of either air or the low oxygen mixture for several hours before the determination was begun. In a number of instances two determinations were made on each rat, one early in the 24-hour period and another at its close. There was a tendency for the oxygen consumption to fall



A B

Fig. 1. Demedullated rat adrenal glands.

A. Three days after operation. Hemorrhagic central area with a partial rim of surviving cortical cells.

B. Eighteen days after operation. Regenerated cortical cells in columnar arrangement, no medullary cells. Note scar from operation.

with the passage of time, but not to such a degree as to affect the group results significantly.

The other methods used have been described previously and were the same except as follows: 1. The rats used were males except in the case of one group which will be noted. This was done because the nitrogen excretion was found to vary less in males. 2. It was previously reported that a mixed salt solution offered as drinking water in combination with a dry diet would maintain adrenalectomized rats in apparent health for periods much in excess of the survival time of untreated rats, and from such rats it was possible to obtain a majority that had gained at least

slightly over their operated weight. Later attempts, however, were not so successful and salt, although prolonging life in many cases by as much as a week did not do so regularly, and most of the animals lost weight from the first. This high morbidity and mortality no longer occurred after several changes were made in the technique, namely: The operation was done on fed instead of fasted rats and lighter anesthesia was used; greater care was also taken to prevent exposure to changes in temperature, the environment being kept throughout the anesthesia and subsequently during the life of the rats as nearly as possible at 27°C. Using these changes but continuing with the salt solution, it was possible to obtain active and apparently healthy rats in their second week post-operative which had gained over the operated weight, this being the criterion previously demanded. On the whole, the rats prepared thus for the experiments here described represented an improvement over previous operative results. To insure against any possibility of adrenal insufficiency they were not used, however, in the second week post-operative but used beginning on the third day, at which time they had fully recovered from the operation and were apparently in vigorous health.

The variation expressed in the tables is the standard deviation of the mean.

EXPERIMENTAL. *Confirmation of previous results.* Since the time of the original report a number of additional intact rats have been exposed to $\frac{1}{2}$ atmosphere. In all of these the characteristic increase in liver glycogen was found to occur. Certain of these deserve special mention. Rats, obviously emaciated and suffering from infection exhibited the usual increase in liver glycogen, as also did rats prostrated in the chamber during the hot days of summer and rats exposed to cold due to accidental failure to control the temperature of the room overnight in the winter. In spite of these and similar adverse conditions no intact rat has failed to show the characteristic increase in liver glycogen. It would not seem necessary to show that exposure to a gas mixture containing 10.5 per cent oxygen in nitrogen would elicit the same response as exposure to $\frac{1}{2}$ atmosphere. Nevertheless this was done with two rats. At the end of 24 hours of fasting during which time they were exposed to the stated mixture the following average values were obtained: Liver glycogen 2170 mgm. per cent, gastrocnemius glycogen 700 mgm. per cent and blood glucose 156 mgm. per cent, such values being within the range of those obtained for intact rats fasted 24 hours at $\frac{1}{2}$ atmosphere (see table 3).

Contrasted with this regularity of carbohydrate increase for intact rats has been the constant failure of its occurrence in adrenalectomized rats no matter how normal in other respects they may have appeared to be. In the section on methods certain improvements in the operation and after care of adrenalectomized rats has been described. Six of these rats had

gained at 3 days post-operative an average of 3 grams, were particularly active and healthy in appearance, eating well, and with rectal temperatures between 37 to 37.5°C. Such animals, it was previously shown, have, when fed, normal carbohydrate stores. But these six after 24 hours of fasting at ½ atmosphere showed low liver glycogen, the average value being 123 mgm. per cent. These observations and some yet to be described make it seem likely that the failure to deposit liver glycogen is a particular result of adrenalectomy and not an effect accomplished through preceding general failure of nutrition.

Urinary nitrogen and ketones. If the endogenously formed carbohydrate had its origin even in part from protein there ought to be a parallel increase in urinary non-protein nitrogen. To determine if this were the case rats were placed in metabolism cages for three days of fasting and the 2nd and 3rd day urines collected and analyzed. One group of intact rats was placed at ½ atmosphere during the second day of fasting and then returned for the third day to atmospheric conditions; another group, serving as controls, was kept at atmospheric conditions for the entire time. Observations were also made on two similar groups of adrenalectomized rats.

The values for urinary nitrogen and ketones on these animals are given in table 1. In order to allow for any lag in nitrogen excretion following the day in the chamber the values have been given for the two-day period.

TABLE 1

Total urinary non-protein nitrogen of rats during the 2nd and 3rd day of fasting; ketone excretion during 2nd day

Milligrams per 100 gram rat

	INTACT			ADRENALECTOMIZED		
	No. of animals	Nitrogen	Ketones	No. of animals	Nitrogen	Ketones
At 1 atmosphere	44	148 ± 4.1	2.2	34	117 ± 4.1	<0.2
2nd day at ½ atmosphere 3rd day at 1 atmosphere	43	192 ± 7.4	8.1	29	118 ± 4.3	<0.2

It will be observed that the intact animals which had been in the chamber excreted in the two day period 30 per cent more urinary nitrogen than the intact control group. The adrenalectomized group in air excreted 21 per cent less nitrogen than did the intact animals and exhibited no increase when placed at ½ atmosphere. Urinary ketones are less in adrenalectomized animals and opposed to the result with intact animals show no increase upon exposure to low oxygen tension.

The number of animals included in each group is necessarily large because of the fluctuations that occur in urinary nitrogen without apparent cause. Ten females were included in each group; for them it was noted that the

variation in urinary nitrogen was larger than for the males and no further females were employed in subsequent experiments.

The failure of adrenalectomized animals either to excrete as much nitrogen as intact animals or to show an increase in nitrogen excretion can not be explained by accumulation of nitrogen in the blood; the average blood NPN of 7 adrenalectomized rats after having been in the chamber for their second day of fast was found to be only 5 mgm. per cent greater than the average value for a like number of intact fasted rats, no abnormally high values being encountered. This apparently would rule out the occurrence of any definite adrenal insufficiency.

Oxygen consumption and respiratory quotient. Determinations of oxygen usage and respiratory quotient were made on three groups of animals, namely: intact animals in air, intact animals exposed to a mixture of 10.5 per cent oxygen in nitrogen, and adrenalectomized animals in air. The results are shown in table 2. The values for intact and adrenalectomized animals are not significantly different. But the oxygen consumption of intact animals exposed to low oxygen tension is definitely lower and the R. Q. higher than similar measurements made on intact animals in air.

TABLE 2

Oxygen consumption and respiratory quotient of rats during the second day of fasting

	NO. OF ANIMALS	OXYGEN CONSUMPTION CC./100 GM. RAT/HR.	R.Q.
Intact at 1 atmosphere	12	123 ± 2.9	0.728 ± 0.0038
Intact at ½ atmosphere	9	100 ± 1.8	0.749 ± 0.0048
Adrenalectomized at 1 atmosphere	4	118	0.722

Effect of hypophysectomy and of demedullation of both adrenals. Hypophysectomized rats, and rats with both adrenal medullae removed as described under Methods were exposed fasting for 24 hours to ½ atmosphere. Table 3 shows the analyses for carbohydrate in these animals; the values previously found for intact and adrenalectomized animals are included for comparison as also those for animals with one adrenal removed and the splanchnic nerve on the other side cut. The hypophysectomized animals had recovered from the immediate effects of the operation and were selected from a larger group because of the absence in them, apart from the failure to gain weight, of any of the signs of pituitary cachexia: they showed no deposition of liver glycogen, although the glycogen of gastrocnemii was normal for fasted animals. The bilaterally demedullated animals at 3 days post-operative gave no carbohydrate response and histological examination of these animals (fig. 1a) showed but a few surviving cortical cells: at 10 to

18 days the glands contained no discoverable medullary cells but showed marked regeneration of the cortex (fig. 1b), and these animals manifested a definite increase in liver glycogen.

TABLE 3

Rats fasting 24 hours at ½ atmosphere

	NO. OF ANIMALS	DAYS POST-OPERATIVE	GLYCOGEN, MGM./100 GM.		BLOOD SUGAR, MGM./100 CC.
			GASTROCNEMIUS	Liver	
Intact	6		806 ± 41	2501 ± 75	125 ± 6
Adrenalectomized	11	14 aver.	559 ± 14	149 ± 20	68 ± 5
Adrenalectomized receiving large doses of cortical extract (see text)	5	3	542	131	
Adrenal medullae removed	4	3	550	188	88
	8	10–18	685 ± 32	1334 ± 252	92 ± 5
Right adrenal removed. Left splanchnic nerve cut	4	7	720	1251	98
Hypophysectomized	6	7–12	527	110	

Effect of cortical extract and epinephrine. Five adrenalectomized rats were given large daily doses of cortical extract for three days post-operative, and then on the fourth day were placed fasting at ½ atmosphere; during this fourth day they received still greater amounts of cortical extract, such being given in two equal doses, the first at the beginning of the fast, and the second within 4 to 6 hours of the end of the fast. No increase in liver glycogen occurred (table 3). Two of these rats received "Eschatin," 10 dog units daily and 20 D. U. on the test day; the remaining three received cortical extract kindly supplied by Dr. J. J. Pfiffner, 15 D. U. daily and 90 D. U. on the test day.

From any of the known actions of epinephrine it would seem unlikely that this substance could mediate an increase in total glycogen; special reasons in favor of this view have been given previously. However, it remained possible that long continued action of this substance might have a different effect than a single dose or injections for short periods. To test this possibility three fasting rats were given 0.02 mgm. of epinephrine subcutaneously every three hours for 24 hours. At the end of this time the average glycogen in gastrocnemii was 389 mgm. per cent and that in the livers 259 mgm. per cent, indicating that although the liver glycogen was slightly higher than that usually found in 24-hour fasted rats, the glycogen of skeletal muscle as judged by the gastrocnemius had fallen more than enough to explain this slight increase.

Phloridzination. If, as the preceding experiments indicate, the adrenals

are concerned in the endogenous formation of carbohydrate, at least in part, from protein, it should be possible to obtain confirmation of this idea by comparing the urinary excretion of sugar and nitrogen in intact and adrenalectomized animals following phloridzination; and if the cortex and not the medulla is the necessary portion of the adrenal, animals with cortical tissue only should resemble intact animals in the extent of sugar and nitrogen excretion. Table 4 shows the results of such observations. The values for urinary ketones are also included. The details of the operations, the method of collecting and analyzing the urine have been described under Methods. The phloridzination was begun 3 days post-operatively in adrenalectomized and ten days post-operatively in the demedullated animals. Autopsy, with histological examination when necessary was done on these as well as on all other animals to assure the success of operative methods. In order to exclude observations upon moribund animals, values obtained within 36 hours of death have not been used in calculating the average values shown.

TABLE 4

Urinary glucose, nitrogen and ketones of phloridzinized rats

Mgm./100 gm. rat/day

	No. of animals	1st day of fasting				Average of 2nd and 3rd day of fasting			
		Sugar	Nitrogen	D/N	Ketones	Sugar	Nitrogen	D/N	Ketones
Intact	14	624	165	3.85	76	478 ± 15	148 ± 5.4	3.18 ± 0.03	229
Adrenalectomized	8	345	114	3.05	23	178 ± 19	68 ± 6.5	2.65 ± 0.06	41
Adrenal medullae removed	7	722	182	3.83	35	487 ± 21	160 ± 7.1	3.12 ± 0.05	126

The results indicate clearly that in the absence of the adrenals the excretion of glucose, nitrogen and ketones is much lessened, but that this diminution does not occur if cortical tissue is present. The results of the first day of fasting are not reliable criteria as they no doubt reflect the extent of food intake on the previous day. The sugar excretion of adrenalectomized animals for the second and third day of fasting and phloridzination was but 37 per cent of that excreted by intact animals; the nitrogen similarly compared was 46 per cent, and the ketones 18 per cent. For the animals with demedullated adrenals the excretion of sugar and nitrogen exceeded that of intact animals; the ketone excretion was however about one half that of intact, but still considerably greater than that of adrenalectomized animals. The D/N ratio was not changed by demedullation, but was lowered significantly by adrenalectomy. It should be noted that whereas

adrenalectomized animals do not tolerate fasting in the ordinary way, they survived phloridzination as well as intact animals. By a comparison with table 1 it will be seen that phloridzinized intact and demedullated animals excrete roughly as much nitrogen per day as they excreted in two days without phloridzin. On the other hand the nitrogen excretion of adrenalectomized animals is not increased greatly by phloridzination and does not exceed that ordinarily excreted by intact animals.

Attempts to increase the urinary excretion of sugar and nitrogen in phloridzinized adrenalectomized animals by injection of 90 D. U. of the cortical extract supplied by Doctor Pfiffner have not been successful. Due to the toxic nature of crude adrenal extracts, animals injected with such preparations have died.

Discussion. There are a number of obvious conclusions that may be drawn from the facts presented. The increase in nitrogen excretion which occurs when intact fasting animals are exposed for 24 hours to ½ atmosphere is more than sufficient to make it possible for the newly formed glycogen to have its origin from protein. The failure of the oxygen consumption to increase in these animals during exposure to low oxygen tensions makes it unnecessary to suppose that part of the increase in nitrogen has resulted from a general increase in metabolism. The lowered nitrogen excretion of animals following adrenalectomy without a concurrent fall in oxygen consumption, and the failure of these animals both to lay down glycogen and to increase their nitrogen excretion when exposed to low oxygen tension suggest strongly that the adrenal is particularly concerned in the conversion of protein to carbohydrate. This last conclusion receives adequate support from the fact that adrenalectomized animals when phloridzinized excrete much less sugar and nitrogen than phloridzinized intact animals. Biasotti and Houssay (1932) have recorded a similar reduction in urinary sugar and nitrogen in phlorhidzinized dogs as a result of hypophysectomy; they found the D/N ratio to be lowered in such animals and to a greater degree than that here recorded for adrenalectomized rats. Long and Lukens (1935a) working with depancreatized cats have found the sugar and nitrogen excretion to be lowered not only by hypophysectomy but by adrenalectomy as well.

Evidence that the cortex and not the medulla of the adrenal is the part concerned comes from several sources; animals deprived only of medullary tissue show the same increase of carbohydrate at ½ atmosphere and when phloridzinized excrete the same amounts of sugar and nitrogen as do intact animals. Epinephrine given during 24 hours in frequently repeated doses was found to lower glycogen stores, and furthermore no known action of epinephrine would suggest it as the responsible agent in such a conversion. Long and Lukens (1935b) also have noted that the amelioration of pancreatic diabetes in cats which occurs as a result of adrenalectomy does not occur if only the adrenal medullae have been removed.

It is recognized that following pancreatectomy or phloridzination, gluconeogenesis is stimulated, the sugar taking origin largely from protein, and it now appears that exposure of rats to low oxygen tension can be placed in the same category as these other two measures, the chief difference being that in exposure to low oxygen the formed sugar is not excreted but is stored at least in part as glycogen. Viewed in this way the present paper constitutes a companion report to that of Long and Lukens (to be published shortly), and of which preliminary reports have already appeared (Long and Lukens, 1934a, b, c, 1935a). These authors have studied the changes in carbohydrate, nitrogen excretion and ketosis which occur as a result of adrenalectomy and hypophysectomy in depancreatized cats. For fuller interpretation of the results here presented and for supplementary discussion, particularly in respect to the relation between the hypophysis and the adrenal gland the reader is referred to their paper.

Ketogenesis has been found to be diminished by removal of the adrenal glands. This is most marked in the experiments with phloridzin. A similar reduction in urinary ketones associated with adrenalectomy and with hypophysectomy has been observed by Long and Lukens (1935a) in depancreatized cats. Rietti (1932) has observed the same result as a consequence of hypophysectomy both in depancreatized and in phloridzinized dogs. The low ketone excretion of adrenalectomized rats and their failure to increase it on exposure to low oxygen tension contrasts (table 1) with the observations on intact animals, these latter having a greater normal fasting ketosis and exhibiting an increase on exposure to low oxygen tension. Although ketogenesis and gluconeogenesis have both been found to be diminished by adrenalectomy it is not intended to imply that these two processes are linked together; indeed, experiments later to be reported from this laboratory indicate that the two effects can be separated one from the other.

The observation that oxygen usage is lowered and the respiratory quotient raised under the same conditions as increase glycogen stores and nitrogen excretion is left without comment at the present time. A variety of explanations suggest themselves but further experimental work is necessary.

The present finding that hypophysectomized animals, like adrenalectomized animals, do not increase liver glycogen at $\frac{1}{2}$ atmosphere is in keeping with the known trophic relation of the hypophysis to the adrenal (Smith, 1930; Evans, Meyer and Simpson, 1932) and also with the similarities of effects following hypophysectomy and adrenalectomy already referred to in this discussion.

On the whole the evidence presented in this paper and in those of Long and Lukens leaves no doubt as to the important rôle played by the adrenal cortex in the conversion of protein to carbohydrate. Although it appears

that conversion from protein is capable of accounting for the increase in carbohydrate, the possible participation of fat is by no means excluded. The mechanism by which the adrenal cortex exercises its influence is at present not clear. It is possible that the hypophysis controls the liberation of some factor in the adrenal gland. This hypothetical substance would appear not to be the substance associated with salt and water metabolism. Other explanations have been considered but a discussion of them is left to await further experimental results.

SUMMARY

The previously reported findings that an increase of glycogen occurs in fasting albino rats when exposed for 24 hours to ½ atmosphere, and that this increase is prevented by adrenalectomy have been confirmed.

Hypophysectomy, like adrenalectomy, has been found to prevent the increase in carbohydrate. The relationship of the hypophysis and the adrenal in this connection is briefly discussed.

A parallel increase in nitrogen excretion, sufficiently large to account for more than the observed increase of carbohydrate in intact rats at ½ atmosphere has been observed; at the same time the oxygen consumption has been found lowered and the respiratory quotient raised. Adrenalectomized rats, although not having a significantly lower oxygen consumption than intact rats, excrete less nitrogen and show no increase at ½ atmosphere.

The sugar and nitrogen excretion of phloridzinized rats has been found much diminished by adrelanectomy.

Rats from which only the adrenal medullae have been removed respond to low oxygen tension and to phloridzination as do intact rats.

Adrenal cortical extract, prepared by the method of Swingle and Pfiffner, has not been found to alter the sugar and nitrogen excretion of adrenalectomized phloridzinized rats nor to raise the glycogen of animals exposed to ½ atmosphere.

The ketosis of phloridzinized rats and of rats exposed to low oxygen tension has been found to be diminished by adrenalectomy.

The conclusion is drawn that the adrenal cortex is concerned in the conversion of protein to carbohydrate.

I wish to thank Dr. J. S. P. Beck of the Pepper Laboratory of the Hospital of the University of Pennsylvania for making the microscopic examinations, and Miss Dorothy Quinn for careful assistance with analyses.

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ON THE PROCESS OF EXCITATION BY BRIEF SHOCKS IN AXONS[1]

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In 1931 (Erlanger and Blair) observations on the behavior of mixed nerves polarized by subrheobasic currents led to the conclusion that fibers might respond to threshold shocks after intervals ranging up to 0.5 millisecond (msec.). This conclusion was confirmed in 1933 (Blair and Erlanger) by direct observations showing that following a threshold shock lasting 0.04 msec., the spike from a single responding fiber developed at the stimulated locus after a period which in various trials with the same fiber ranged from 0.16 msec. up to 0.41 msec. The present paper is concerned with the nature of the process which develops in the interval between the removal of the imposed potential and the appearance of the axon spike.

METHODS. As a routine observations have been made on the single, most rapidly conducting axon in the distal attenuated portion of the sciatic-phalangeal nerve preparation of *Rana pipiens* previously described (Blair and Erlanger, 1933). The spike of the action potential has been recorded by a cathode ray oscillograph after amplification up to 2,000,000. The stimuli were shocks from Porter induction coils with removable cores (fig. 1). The coils were iron-cored only when it is so stated. The secondaries in all cases were home and the strength of the shock was controlled by precision resistances in the primary circuit. The make shock, usually below threshold, was disregarded. Experiments were conducted at room temperature except when otherwise stated. A rate of stimulation (usually 30 per min.) below that producing cumulative effects was used. Hg-HgCl electrodes were employed exclusively. By means of the modified bridge balancing circuit of Bishop it has been possible to reduce the shock artifact to an extent that permitted recording of the initiation of the threshold unconducted spike of a single fiber at the electrode which was at the same time the cathode of the stimulating circuit and the ground lead to the amplifier.[2]

[1] This investigation was aided by a grant from the Rockefeller Foundation to Washington University for research in science.

[2] When a single electrode is the cathode of the stimulating circuit and at the same time the effective ground lead to the amplifier, stimulation should occur on the side

EXPERIMENTAL PROCEDURE AND RESULTS. A. *Spontaneous variations in axon threshold.* Irregularity of response of axons to currents which just stimulate has been referred to in previous papers. When the stimulus repeated at the rate of 30 per minute is gradually increased from a subthreshold level, a strength will be reached which will result in, say, one response in ten to twenty trials. In round numbers a further increase of 10 per cent in the stimulating voltage is required to change the probability of response from this low figure to a very high value. We have arbitrarily

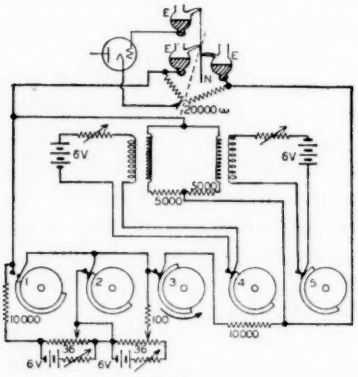

Fig. 1

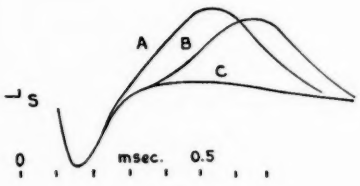

Fig. 2

Fig. 1. Stimulating and balancing circuit used for study of axon spikes at the locus of stimulation. The effective balance of the bridge is indicated by the dotted line. N = phalangeal nerve. E = calomel half-cell electrodes. All cams are mounted on the same shaft. Keys 1 and 2 impose constant currents on the nerve from two independently variable potential sources; key 3 breaks both circuits. Keys 4 and 5 interrupt the primary currents of two induction coils.

Fig. 2. Superimposed tracings of serial records obtained from the stimulated locus. Description in text.

defined threshold as the current strength which stimulates with approximately half of the trials. Usually the responses do not appear in any

distal to the recording circuit, and the spike should reach full value only after conduction across the electrode. The conduction time might be expected to add to the shock-spike time. The effective width of the electrodes does not exceed 2 mm., and, with the average fiber employed, the time required for the impulse to travel this distance is less than 0.08 msec. This maximum value is small in relation to the shock-spike time. Since a spike begins to record when it is three segments distal to the lead (probably in excess of 3 mm.) and currents usually stimulate at the node nearest to the cathode, we feel that no significant part of the delay can be assigned to conduction. Except for the systematic error associated with determinations of the time of onset of a process which starts at an accelerating rate, the determinations may be taken to represent the time required for the response to appear at the stimulated point.

predictable manner (Blair and Erlanger, 1933); the threshold variations appear to be random within the range mentioned. In a very few cases the responses have occurred with a fairly predictable sequence. In one exceptional case, an axon stimulated by uniform shocks at the rate of 60 per minute responded to five successive shocks and failed to respond to the next five. By adjustment of the current strength, cycles of 5, 6 or 7 responses, followed by an equal number of failures, could be obtained. Increasing the rate of stimulation while keeping the strength at "threshold" reduced the number of responses in a cycle. Similar cycling of responses can often be approximated in normal nerve by a frequency of stimulation of 15 or more per second. We are inclined to assign the termination of a series of responses to fatigue, the first response of the series to recovery from fatigue, and the continuance of the series to a long supernormal phase or treppe. It is possible that phase differences develop between the rhythmic stimulation and the essentially rhythmic processes of recovery from activity and the development of fatigue, and thus account for the apparently random appearance of responses to repeated uniform stimuli. As an alternative hypothesis these variations in threshold may be referable to a more rapid variation in excitability seen when an hyperexcitable axon responds repetitively (to be considered in another connection).

B. *Configuration of growth of excitation during the response time.* 1. *Spontaneous variations in response time and their relation to strength of stimulus.* In a number of experiments in the present series the escape of the threshold shock has been suppressed by balancing to an extent permitting a significant reading of the time of the effective initiation of the spike. In all cases there is a variable delay between the beginning of the threshold shock and the appearance of the spike amounting to as much as 0.5 msec. The results of a typical experiment are shown in figure 2. The three curves are superimposed tracings of records taken with the same strength shock under identical conditions. *S* marks the beginning of the shock artifact. In *A* the spike appeared 0.22 msec. after the shock, in *B* 0.36 msec., a difference of 0.14 msec. The crest play can be made out more clearly; it measures 0.17 msec. This is the maximum play recorded in this experiment; even wider spontaneous variations were observed. Curve *C*, obtained when the axon, due to a spontaneous change in excitability, failed to respond, shows the distortion of the base line due to the shock escape.

The spontaneous variations in threshold discussed above make it possible to attribute the variations in response time to a developing excitation of constant configuration that attains threshold level at different times. When the excitability is high, threshold is attained early and the response time is short; when low, threshold, if attained, will be reached late, and the response time will be long. On the basis of this interpretation the variation in response time is an expression of the time during which the excita-

tion changes by an amount equal to the spontaneous variation in excitability. In round numbers this spontaneous variation amounts to 10 per cent and the threshold response time varies roughly over a range amounting to half the maximum response time. It appears, therefore, that the curve of developing excitation reaches 90 per cent maximum in about half the time for the attainment of maximum. With shocks just strong enough to produce a response at every trial, the play can just be demonstrated; in this region excitation rises so rapidly that the time required for excitation to change 10 per cent is very short. With stronger shocks and consequently earlier responses the time required for a change of 10 per cent can hardly be demonstrated. The indicated curve of excitation, one rising rapidly during the shock, decelerating to reach 90 per cent in roughly 0.15 msec. and maximum in 0.3 msec., is probably approximately correct, but if there be rapid spontaneous variations in excitability, then a rising excitability might cause a response after the maximum excitation had been attained, and the time of the maximum indicated above may be too long.

2. *Relation of stimulating voltage to response time.* As mentioned previously the mean response time may be modified by changing the stimulating voltage. At the stimulated locus this relation cannot be followed quantitatively because higher voltages *a*, stimulate less excitable fibers whose spikes complicate the record; *b*, shorten the response time so that the spike develops in the unbalanced portion of the shock escape, and *c*, stimulate regularly so that distortion of the base line by the shock escape cannot be evaluated. Qualitative observations show that a slight increase above threshold voltage produces a marked decrease in response time, and that further increases produce progressively less effect. These observations support the preceding interpretation: the curve of excitation rises sharply at first, and then more slowly to reach a maximum 0.2 to 0.4 msec. after the start of the shock.

3. In an effort to secure *indirect evidence on the effect of stimulating voltage on response time*, the growth of excitation was opposed during the shock-spike time by a shock of opposite sign to that of the threshold stimulating shock (cored coils) and the spike was recorded from the stimulating electrode. When the cathodal stimulating shock was fixed at threshold and the strength of the opposing anodal shock required to prevent the attainment of threshold at various times in the shock-spike interval was determined, the lower curve of *A*, figure 3, was obtained. If it be assumed 1, that changing the anodal voltage changes quantitatively the amount of suppression at any given time without otherwise modifying the curve and 2, that the two shocks sum without further modification, then the reciprocal of the voltage required to prevent the response, plotted against the time it may operate prior to the attainment of threshold, should express the temporal configuration of the development of the anodal effect. The

result is shown in *B*, figure 3 (dots). The broken portion of the curve is drawn on the assumption that the appearance of the spike coincides with the time at which a shock of infinite voltage would be required to inhibit a threshold response.

In the same experiment the opposing anodal shock was fixed at a value sixteen times the strength required to abolish the response when delivered simultaneously with the threshold stimulating shock. It follows that no response to threshold stimulation could occur after the anodal shock of fixed voltage had produced one-sixteenth of its maximum effect. The voltage of the stimulating shock required to produce a response with various separations was determined. The results are plotted as the upper curve of *A*, figure 3. As shown above, after a period of inception, an-

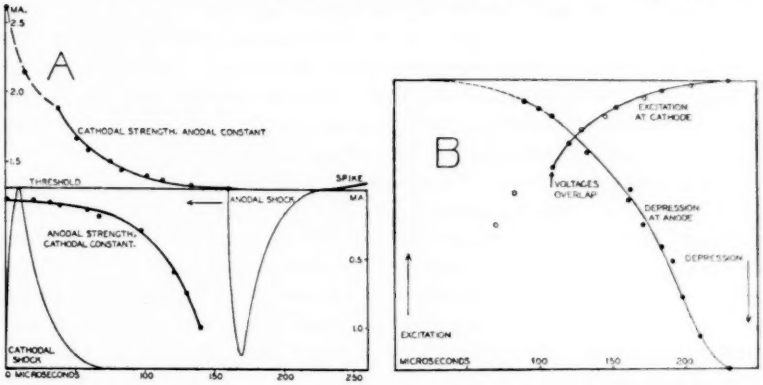

Fig. 3. A. Curves showing the temporal summation of the effect of opposing shocks. Explanation in text.

B. The derived curves showing the development of excitation and of depression.

odality rises so rapidly that it would serve to prevent the attainment of threshold after this nearly constant period. If the assumption be made that threshold is attained at the time of appearance of the spike, the inception period is 0.07 msec. and no response can follow the anodal shock after a longer delay. Therefore, for wide separations, the interval between the cathodal and anodal shocks, plus the nearly constant inception period of the anodal effect, will give the shock-spike time approximately. When the voltages begin to overlap, and the cathodal shock has been increased, the anodal shock no longer is able to prevent a later increase in excitation, and the broken portion of the curve is simply an expression of the algebraic summation of applied voltages and without significance in this connection.

When the reciprocal of the cathodal strength is plotted against response time, or the attainment of threshold determined as discussed above, a

portion of the curve of the development of cathodal excitation is obtained (circles, *B*, fig. 3). While this part of the curve of excitation approaches its maximum at a slower rate than the curve of depression, it is probable that both have similar general configurations, starting after a period of inception, increasing rapidly, and then slowing as a maximum is attained.

4. *Absolute latency.* As the axon spike begins at a relatively slow rate, its start cannot be significantly marked in terms of a few microseconds, and we cannot with assurance relate the beginning of the axon spike to the curves of excitation derived from the algebraic summation of opposing shocks. As determined, the asymptote of the curve of voltages required to suppress threshold excitation, or the time at which excitation had reached a stage where it could not be overcome by an opposing voltage of any strength, precedes the response by approximately 0.070 msec. If there be a latency between the attainment of threshold excitation and the appearance of the response, it does not exceed this figure.

Discussion. There are at least three possible processes accounting for the curve of rising excitation which reaches a maximum in 0.2 to 0.4 msec. following a brief shock: 1, Utilization for shock stimulation; 2, time required for the development of an intermediate local excitatory process; and 3, distortion of the applied potential by the complex electrical circuit of the nerve resulting in the attainment of a delayed maximum potential across the excitable interface. These possibilities will be considered seriatim.

1. *a.* Cathode ray oscillograph records of the shock show a maximum demonstrable duration, under the conditions of the experiment, of 0.04 msec. If the utilization period involved the entire duration of the shock, this period could account for only one-third of the minimum shock-spike delay.

b. Utilization time should vary directly as the duration of the applied potential if the strength is kept at threshold. In a typical experiment an axon showed an average threshold response time to a shock from a coreless coil of 0.238 msec.; when the shock duration was effectively doubled by inserting the iron core, the average response time increased to 0.264 msec. This slight increase is of the order of the increase in duration of the shock, and demonstrates that the response time is not a simple expression of shock duration.

c. To control the possibility that there might be a residuum of the shock which, though not demonstrable with the cathode ray oscillograph, could contribute to the stimulation of the axon, the secondary was short-circuited by a synchronized key. The make contact time of the key was not sufficiently constant for a quantitative statement of the interval between the start of the shock and the closing of the shunt, but no effect on the stimulating strength of the shock could be observed until the interval was so short that the short-circuiting key suddenly reduced the amplitude of the

shock escape. The shunt probably operated in this case around 0.010 msec. after the initiation of the shock; and certainly the current was allowed to flow a very much shorter time than the observed duration of the shock. It follows from the above experiments that no significant fraction of the shock-spike time, or of the continued growth of excitation, can be referred to utilization time as ordinarily defined.

2. If there be a local response of nerve, the rate of development up to propagating intensity must be determined by the intensity of the applied voltage. The excitation level attained during the effective utilization period of about 0.01, or an outside maximum of 0.04 msec., must determine the amount and rate of increase up to maximum excitation some 0.26 to 0.3 msec. later. The continued growth of excitation during 95 per cent of the time required to attain a maximum is not directly dependent on the applied potential; but, during this period, excitation can be prevented by a sufficiently strong opposed potential, as demonstrated above.

If the local excitatory process represents a reaction which, once initiated, develops independently of imposed conditions, one might expect this release of energy to be accompanied by some electrical sign. Earlier experiments have shown that any potential in nerve which can be dissociated from the stimulating potential always takes the form of a conducted spike. We may add to this evidence recent findings which show that with threshold shocks the response time may vary spontaneously through a range as wide as the minimum delay with no demonstrable change in the balanced artifact of the stimulus preceding the spike. If there be a local response of nerve to subthreshold shocks, no associated electrical potential can be demonstrated by a method which would show a potential amounting to 2 per cent of the spike.

3. The character of the local excitatory process discussed above can be accounted for more satisfactorily on the assumption that the growth of excitation represents the growth of potential across the responding interface through the complicated circuit of the nerve as a whole. In recent years Ebbecke (1927), Bishop (1928), Schaefer and Schmitz (1934) and others have suggested electrical circuits as models to account for the behavior of nerve. The growth of excitation to a late maximum can be satisfactorily explained on the basis of our experiments upon the assumption that the shock charges a large leaky capacity which, in reference to the applied potential, is in parallel with a resistance and a smaller capacity of the polarizable responding interface; that, after the removal of the applied potential, the large capacity discharges through its leak and also through the responding mechanism; and that the maximum potential at the latter interface is attained at threshold response time.

With this system 1, the configuration of the rise of potential from a short shock across the small capacity has essentially the same configuration as

the rise of excitation as determined by the play in response time and by the change in response time produced by increasing the applied potential; 2, two opposed shocks, each distorted to the same degree, sum algebraically to produce potential changes across the small capacity which agree essentially with excitation changes resulting from the summation of effects of opposed shocks; and 3, the configuration of the potential rise across the small condenser is not significantly modified by changing the shock duration by the insertion of an iron core.

All the experimental findings on nerve cited above can be satisfactorily explained on the assumption that the applied shock is distorted by the complex electrical system of the nerve, and that the "local excitatory process" is the slow rise of potential to a late maximum at the responding element.

SUMMARY

1. Evidence is presented indicating that most if not all the latency of response of an axon to a short shock can be accounted for by referring it to a delayed rise of the stimulating potential at the responding locus.

2. Variation in response time under constant conditions appears to be a reflection of spontaneous variations in threshold operating on the relatively level part of the curve of excitation.

3. If there be a period preceding the spike when the "local excitatory process" cannot be antagonized by an opposing potential, its duration does not exceed 0.01 msec.

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ON EXCITATION AND DEPRESSION IN AXONS AT THE CATHODE OF THE CONSTANT CURRENT[1]

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In a preceding paper (Blair and Erlanger, 1935) the conclusion was reached that excitation determined by a short shock behaved as if it were a delayed rise of potential across the responding interface, and that the propagated response was initiated essentially at the time that excitation attained threshold value. In this paper the growth of electrical excitation is followed by relating the voltage of long constant currents to the time of appearance of the spike at the stimulated locus. The results are correlated with data derived through determinations of strength-duration relations, and through a study of certain effects of the addition to subrheobasic currents of threshold constant currents and brief shocks varied temporally with respect to the make of the former.

METHODS. The preparation and methods were as described in the preceding paper. The nerve could be stimulated at the recording electrode by independently variable shocks from two induction coils and by two constant currents from different sources of potential. Because of the lower voltages and longer times involved, the make artifact could readily be separated from the axon spikes appearing at the stimulating electrode by the method shown in figure 1 of the preceding paper. The make-spike time may be reduced by 70 per cent without serious complications from a change in the base line due to the make artifact.

PROCEDURE AND RESULTS. Using a long rectangular current at threshold, a spike from a single (the most irritable) axon appears at the stimulated locus after a delay which varies from trial to trial. For the fastest fiber in the phalangeal nerve at room temperature the make-response time usually plays with different trials between the limits of about 1 and 1.5 millisecond (msec.). When the stimulating voltage is increased, the spike moves earlier and the range of spontaneous play at any given strength is reduced. With a further increase in voltage the spike moves still earlier, is stabilized temporally and usually spikes from axons of lower excitability appear later.

[1] This investigation was aided by a grant from the Rockefeller Foundation to Washington University for research in science.

With still higher voltages, the whole complex approaches the make artifact and the later spikes become more nearly superimposed on the initial spike. With high voltages (2 x rheobase) the whole spike complex becomes effectively superimposed and moves forward into the make artifact.

There are certain features of the method that limit the accuracy of measurement. 1. The spontaneous play of response time when the axon is stimulated by currents near the rheobase would render the qualitative selection of a statistical point precarious. Instead we have endeavored to define the limits of spontaneous variation. This necessarily spreads the curve at low voltages. In an endeavor to insure securing a comparable range for each determination, we have in practice marked on the face of the tube the time of appearance of the spike in 100 successive trials for each low voltage reading, and have plotted the limits of the play. The response time play complicates only the lower voltage range. 2. The phalangeal nerve contains several large fibers which are stimulated in the higher voltage range. Until the spikes become effectively superimposed, the fiber with the highest excitability has the shortest response time for a given voltage, and it has been assumed that this relationship is maintained after the separate spikes become indistinguishable. 3. Axon spikes rise slowly at the start, and increased amplification therefore brings the first demonstrable part of the spike earlier. When a number of spikes are effectively superimposed the starting time may be read too early in respect to the criterion used for the single lower spike. By the method employed time can be read to 2 per cent of the longest response time.

In figure 1 the bold line represents a typical curve of the relation between the intensity of a long constant current and the interval between the make of the current and the appearance of the spike at the stimulated locus. At rheobase the response appears with various trials in the range between 1.26 msec. and 1.83 msec. As explained in a previous paper, this variation in make-spike time is probably a reflection of a spontaneous variation in excitability resulting in the attainment of an excitatory process to threshold at various times. That the play is not an expression of a shift in the locus of stimulation with a consistent difference in the rate of growth of excitation from point to point, is indicated by the behavior of a spike from a depressed axon. Because of the depression the segment notches were in evidence (see Erlanger and Blair, 1934). In spite of a wide shift in response time, the order of entrance of the notches remained the same; the spike, in other words, always started from the same segment. If the assumption be made that stimulation occurred in the immediate region of the cathode, the observation indicates that the stimulated segment had a variable threshold and that as a result the time required for excitation rising on a uniform curve to reach this shifting threshold also varied. Increasing the stimulating voltage reduces the spontaneous play of response time and

shortens the mean response time. Further increases in voltage essentially eliminate the spontaneous shift and reduce the make-response time in a decrementing manner.

In the preceding paper the conclusion is reached that excitation following a short shock develops as though the shock charged a condenser which

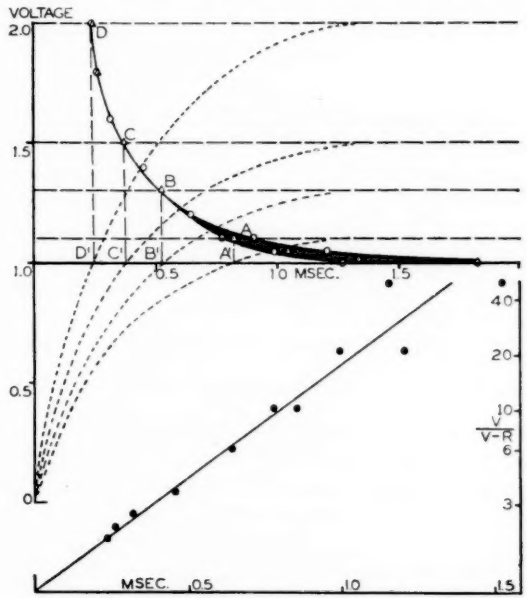

Fig. 1. Upper curve. Circles; the effect of the stimulating voltage on the time between the make of the constant current and the appearance of the spike at the stimulated locus. Triangles; calculated response times. If the effective resistance-capacity product of the nerve is 3.42×10^{-4} then an applied voltage A (broken line) should rise across the capacity along the lower dotted curve and stimulate when the voltage reaches rheobase at A'. Similarly when D ($2 \times$ rheobase) voltage is applied the potential on the capacity should rise along the upper dotted curve reaching rheobase at D'. Points determined in this manner (triangles) and thus plotted in the graphic form suggested by Bishop (1928a) conform to the experimental curve.

The lower curve shows graphically that the voltage-response time curve over the greater part of the time range follows the formula given in the text.

then discharged through a resistance and the capacity of the responding interface to reach threshold after the applied potential has been removed. Since the large condenser which acts as a reservoir must receive its charge during a short shock (approximately 0.04 msec.), the hypothesis requires that the resistance in the charging circuit be very small. With this electrical

system, the large capacity, charging to the applied voltage almost immediately, should not materially affect the growth of excitation from a continued constant current, and the voltage-response time curve should fit the basic formula which underlies the usual mathematical treatment of strength-duration relations,

$$V = \frac{R}{1 - e^{-\frac{t}{k}}}$$

where V = applied voltage, R = rheobase, t = time and k = a constant. The concept signifies that an applied e.m.f. produces a potential difference at the polarizable responding interface which develops at a rate determined by the series resistance through which the current must flow and by the effective capacity of the condenser which must be charged. When the potential difference reaches threshold the nerve responds. When the applied potential is increased to 2 x rheobase, the response occurs when the condenser has reached half charge.

As suggested by H. A. Blair (personal communication) a curve following this formula should result in a straight line when $\log \frac{V}{V-R}$ is plotted against time. That this relation holds is shown in the lower curve (fig. 1).[2] From any point on the experimental curve it is possible to derive k (the resistance capacity product), a constant which determines the rate of rise of potential in the system. Knowing k, which in this case is 3.42×10^{-4}, and the rheobasic voltage, it is possible to determine the time required for any applied potential to reach threshold voltage. Due to the dispersion of response times at voltages near threshold the results are not entirely unequivocal, but in 32 experiments no consistent deviations from curves of this type have been noted. In two cases the make-response time curves did not fit the formula. When conduction is involved the curves are no longer simple, presumably because the higher voltages spread the locus of stimulation, thus reducing the conduction time. Significant conduction time from the stimulated locus may have been the reason for the two atypical curves.

The asymptote of the curve derived by extrapolation (the time required for stimulation by an infinite voltage) in a number of experiments was 0; in others considerable divergence in both directions has been observed. This divergence is probably an expression of experimental error and therefore it is concluded that if there be any delay between the attainment of

[2] Using a different approach Schmitz and Schaefer in 1933 reached essentially the same conclusion but modified it in 1934 when their technique was extended to include a study of the discharge of nerve.

threshold and the appearance of the spike, it is less than the limit of error of the method.

At room temperature the constant, k, is usually around 3.5×10^{-4}. In one case cooling to 8° increased this value to around 2×10^{-3} without changing the configuration of the curve.

Observations have shown that with rheobasic voltages excitation never reaches threshold after the break of the constant current. As the applied potential is increased responses may occur after the break when the duration of the current is reduced so that responses occur only occasionally. With higher voltages and briefer currents responses occur consistently after the break of the current. This break-spike delay would not be demonstrable with a submaximal multifiber spike (see Bishop, 1928a) because

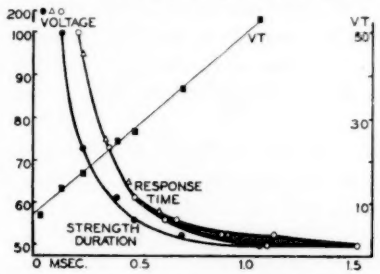

Fig. 2. Curves showing the relation between strength and duration (dots), and strength and response time (circles). The time between the opening of the stimulating current and the appearance of the spike is vanishingly small at rheobase, increases rapidly as shown by the separation of the curves, with higher voltages, and appears to become very nearly constant. The triangles represent the calculated response time values. The vt curve (squares) indicates that the strength-duration curve has the usual hyperbolic configuration.

there would always be a number of contributing fibers responding to threshold stimulation with no break-spike interval.

Figure 2 shows the result of a typical experiment comparing strength-duration curves with strength-response time curves. For each voltage the response time was determined with a long constant current, and then the duration of the same current which just failed to stimulate. The circles represent the experimental points for response time; the triangles mark the calculated response time curve, and the dots, the strength-duration curve. The Weiss law curve, $v \times t$ (squares), indicates that the strength-duration curve has the usual hyperbolic form. The curves show that the interval between the break of the current and the appearance of the spike at first increases very rapidly with the voltage and then apparently becomes very nearly constant. In effect, when a strength-duration curve is plotted, the *Hauptnutzziet* agrees with the response time, but all other utilization times

are too short by an amount equal to the break-spike interval. As the response moves into the increasing make artifact (with voltages above 2R) the range of the curve is not sufficiently great to justify deciding as to whether or not the break-spike interval becomes a constant.

Since the brief response times resulting from stimulation with high voltages cannot be determined, it is impossible to trace the configuration of the curve for the higher voltage range. While it is probable that distortion of the simple curve occurs in this range, we believe that the simple relation obtaining between voltage and response time as determined over a range of 85 per cent of the maximum response time justifies the conclusion that constant current excitation rises along a curve having essentially the shape of the rise of voltage on a condenser which is charged through a resistance. Given the rheobase and the response time for any higher voltage, it should then be possible to express the response time curve, and the curve of the development of excitation, over the greater part of the time range (but not of the voltage range) without the introduction of any arbitrary constant.

A large amount of work has been done on the change in threshold during a subrheobasic constant current as tested by induction shocks. The curves show that following the make the threshold decreases for about 0.5 msec., remains effectively unchanged for about 0.5 msec. and then rises along a decrementing curve (Erlanger and Blair, 1931). The rise in threshold must represent either a decline in excitation or a decrease in excitability. On the assumption that excitation is an expression of the voltage across the irritable interface, Bishop (1928b) has assigned the rise in threshold to a decrease in current flow due to a large capacity effectively in series with the stimulating circuit. This conclusion does not seem warranted because 1, excitation, as has been shown above, develops along a curve determined by a simple capacity-resistance system, not by a double condenser system; 2, the response-time play to a rheobasic current, and the analysis of the changes in threshold produced by a subrheobasic current show that after the attainment of an effectively steady maximum of excitation there is a delay preceding the rise in threshold, and this dissociation of the processes of excitation and depression cannot be simulated by a double condenser system; and 3, when the excitability of a nerve is determined by a stimulus placed 20 msec. after the make of the constant current, high polarization voltages result in an elevation of threshold above the normal level (see below): a double condenser system should never result in an elevated threshold. The increased threshold, therefore, probably represents a decrease in excitability, rather than a decrease in the amount of excitation. Cathodal depression seems to be a change in the reactivity of the nerve resulting from an applied current of any strength and does not seem to be related to the properties of the nerve as an electrical system.

Curves of cathodal depression as derived by a testing shock are deformed by the fact that the shock-spike time during polarization may vary through a range of from 1 to 3. As has been shown, excitation by a constant current develops along a definite curve to a reproducible maximum, and it seemed, therefore, that the proper way to determine the amount and temporal development of cathodal depression produced by a subrheobasic current should be to ascertain the change in rheobase. Figure 3 is the plot of an experiment in which a cooled nerve was polarized by a current 90 per cent of rheobase starting at zero time. At different times after the make a second constant current (*en echelon*) was applied to the nerve and the voltage of the latter necessary to produce a response determined. When the two makes were simultaneous the response time varied in different trials as shown on the lower line of the figure. No change in the prob-

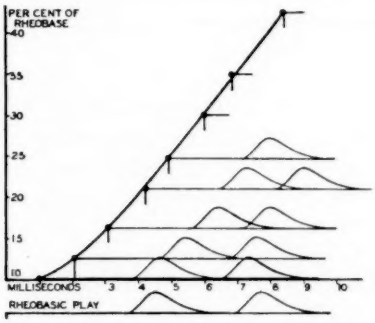

Fig. 3. The decline in effectiveness of a 90 per cent rheobasic constant current starting at 0 time, as tested by a second constant current (*en echelon*). The dots represent the starts of the testing currents and the conventionalized spikes show the time of appearance and range of the response at threshold. The limits of response times to rheobasic currents are shown on the bottom line. T = 10°C.

ability of response or in the response pattern occurred until the starts of the two currents were separated by 1 msec., when it became necessary to increase the voltage of the second echelon 0.2 per cent in order to reach threshold which we have defined as the voltage which produces a response to half the trials. As the second make was moved later the probability of response was further reduced, necessitating further increases in voltage, the longest make-response time remained essentially unchanged and the play of the response time was reduced by a gradual drift of the early responses into later positions. No change occurred in the time of the latest responses until the starts of the constant currents were separated by more than 3 msec. With greater separations of the echelons the threshold rose more rapidly and, with the falling of the spikes into positions later than the latest rheobasic spikes, the play became wider again. Later the curve of

threshold becomes less steep, and although wide separations have not been followed, the work of Bonnardel (1934) indicates that the nerve eventually reaches an equilibrium state.

When the two currents are superimposed and adjusted to rheobase, the probability of response diminishes as the testing current is moved later, but responses do not fail completely as long as the excitation from the testing current attains an effective maximum before the time of the latest rheobasic response. A current of this strength never produces a response later than the latest rheobasic response. Since the probability of response decreases with the decrease in the play of response time, the impression is gained that the decrease in number of responses is largely referable to the reduction of the period during which the constant current excitation might sum effectively with peaks of spontaneous variations in excitability; there is a statistically decreased probability of the two effects adding to produce

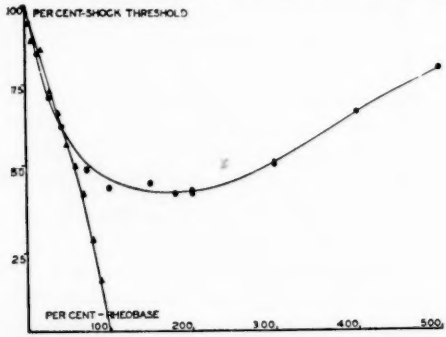

Fig. 4. Immediate and late effects of constant current strength on shock thresholds. See text.

a response of the axon. Such distortion as occurs from this source, will be in the direction of an apparent reduction in the time of onset of cathodal depression; therefore the conclusion may be drawn with assurance that excitation determined by the first constant current reached an effective plateau, which in this case lasted at least 1 msec. and probably longer, before a reduction in excitability occurred. The effect of separating the makes of the two currents on the duration of response time play, and the relatively fixed maximum time between the make of the conditioning current and the appearance of the response, indicate that the plateau of excitation has the effective duration of the rheobasic response time play, and that the maximum rheobasic response time is limited by the appearance of a degree of cathodal depression that is in excess of the spontaneous variations in threshold. In this case the earliest rheobasic response occurred in slightly under 4 msec.; the time of onset of significant depression therefore was certainly not earlier than 5 msec. and probably as late as 6.4 msec.

The experiments with echelon currents supply information relative to the temporal growth of cathodal depression. To gain information on the relation of depression to the strength of polarizing voltages two points on the temporal curve (fig. 4), one near the plateau (triangles), and the other 20 msec. later (dots), were followed by means of changes in shock threshold. Since cathodal depression grows relatively slowly, changes in shock-spike time would not significantly distort the curves. Increasing the constant current reduces the late threshold, but the effect of additional equal increments of current becomes less as the total voltage is increased. Indeed, for the higher voltages (above 2 x R) the threshold begins to rise. The whole effect appears to develop along a smooth curve.

Since in the weak polarizing range the early threshold is higher than in the late, the early shock, in this experiment, was apparently delivered before the maximum excitation was attained. For this temporal separation equal added increments of polarizing voltage sum with no loss in effectiveness. The experiment properly performed indicates that in the initial stages currents up to 1 rheobase produce no depression; while 20 msec. later cathodal depression results in a progressive decline in effectiveness of equal increments of polarizing voltage.

If, as Hill has assumed (1932), polarization results in the passage of cations through the nerve membrane, and if excitability is related to the ratio of the concentration of cations inside and outside, then the delay in the appearance of cathodal depression could be attributed to the time required for the ions to traverse the membrane, the rapid growth that then begins, to the appearance of the ions on the outside, and the slow attainment of the maximum to a correspondingly slow attainment of a new equilibrium state under the effect of the polarization.

Due to the changes which occur during the variable shock-spike interval the change in shock threshold during a subrheobasic current presents a distorted picture of the rise of excitation and of the onset of depression. In an effort to evaluate such distortion a nerve was polarized by a constant current at 90 per cent of the rheobase and the threshold to a shock and the corresponding shock-spike times were recorded from the stimulated locus at various times after the make. These data were also compared with the normal rheobasic response times and shock-response times in unpolarized nerve. The results are shown in figure 5. When the shock is placed early in the constant current the threshold is high and the response time is long. As the shock is moved later the threshold decreases rapidly and the shock-spike time is reduced. At about 0.8 msec. the shock threshold reaches a low level which is maintained for about 0.5 msec. In the region of the plateau most of the responses appear within the range of the rheobasic play. As explained for echelon constant currents, the probability of response decreases as the range of response play to the shock is reduced,

but a shock which is threshold for the plateau stage never causes a response later than the latest previously determined rheobasic response. Possibly as a result of the decrease in the time during which the shock excitation might sum with peaks of spontaneous excitability changes to produce a response, the rise in threshold develops gradually and then, at the time that the shock response appears later than the time of the latest rheobasic spike,

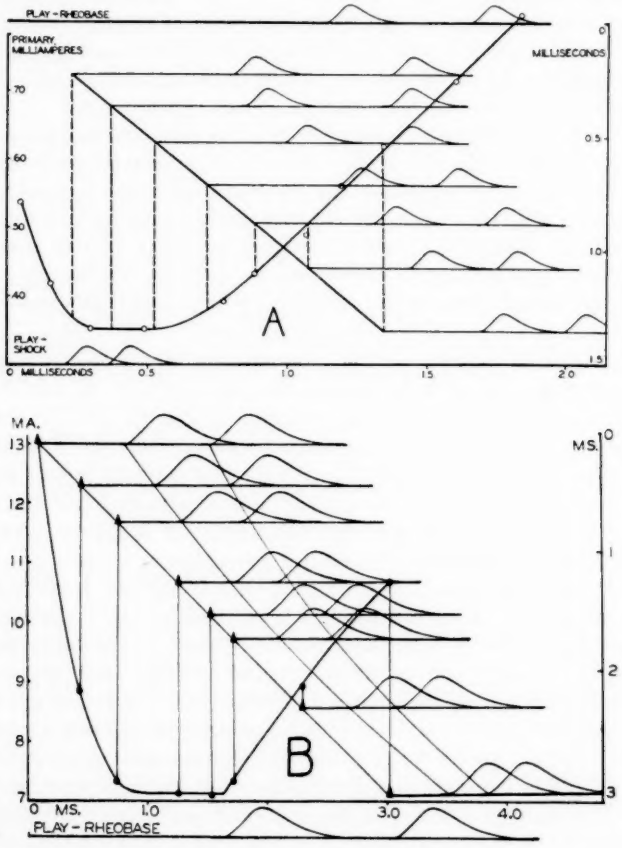

Fig. 5. A. The change in threshold produced by a constant current 90 per cent of rheobase as tested by induction shocks (circles). The conventionalized spikes on the top line indicate the rheobasic response time and its play, on the bottom line, the shock-spike time and its play in unpolarized nerve. The other spikes indicate the shock-response time and its play for various points on the threshold curve.

B. A similar experiment with the threshold curve drawn between points, so as to conform with the consistent results obtained from multifiber responses.

the rate of depression becomes maximum. As with the echelon currents, the curve beyond the range of the figure becomes less steep and approaches an equilibrium level. While of little value in determining the configuration of the changes in nerve produced by subrheobasic currents, this analysis clearly indicates that following the make of a constant current excitation rises rapidly to a maximum level which is terminated by the effective appearance of a second process, that of depression.

SUMMARY AND CONCLUSIONS

1. Excitation produced by a constant current as determined by the relation of voltage to the make-response time over the range investigated (85 per cent of the maximum response time) develops along a curve which agrees, within the limit of error of the method, with the development of voltage on a condenser which is charged through a resistance.

2. This fact, considered in conjunction with previously reported conclusions, indicates that the local excitatory process is a growth of the stimulating potential across the polarizable responding interface.

3. Strength-duration relations as an index to the growth of the local excitatory process are complicated by the fact of a growth of excitation to a maximum continuing after the break of the shorter constant currents. It is for this reason that strength-duration curves do not exactly fit the simple condenser formula in the range to which this method is applicable.

4. The effectively steady state of maximum excitation shown by the wide temporal play of the responses to rheobasic currents and by the constant thresholds found when testing with a second constant current, or with a shock, is terminated by the effective entrance of a second process of cathodal depression, or accommodation.

5. The temporal configuration of the curve of depression is discussed in relation to the concept that depression from a constant current is caused by the passage of cations through the nerve membrane.

We wish to thank Dr. H. L. White for assistance in the mathematical phases of these problems.

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OBSERVATIONS ON REPETITIVE RESPONSES IN AXONS[1]

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In the normal discharge of its functions the tangible messages a peripheral nerve fiber transmits, be it from the nerve centers to the periphery or from the sense receptors to the centers, practically invariably consist of a longer or shorter series of rapidly repeated action potentials; yet such relevant information as we possess on this subject all indicates that this intermittent activity of the fiber is determined by an uninterrupted liberation of energy in the sending station. The clearest illustrative case, perhaps, is that of light reception: the retina when steadily illuminated develops in effect a continuous potential (Hartline, 1928), whereas the optic fibers at the same time transmit intermittent impulses to the brain (Hartline and Graham, 1932). To mention just two other examples, a steady tension on a stretch receptor causes the nerve fiber connected with it to discharge repetitively; and the slow rhythmical potential changes in the brain of *Dysticus* each causes a burst of action potentials to travel along the fibers innervating the respiratory muscles (Adrian and Buytendijk, 1931). To all appearances the sending stations determine the repetitive discharges along the fibers by developing a continuous or slowly changing potential drop along, and consequently a continuous current flow through, the latter.[2] If so, it should be possible, through the use of applied continuous potentials, to duplicate in nerve fibers the repetition pictures that are determined by end organs.

But the usual response given by excised frog's nerve during the action on it of a rectangular constant current at, or above, threshold strength consists of a single action potential developing after the lapse of a brief utilization period. Failure of the nerve to yield more than one action potential under these circumstances is generally ascribed to the intervention of cathodal depression, though other explanations have been proposed (Bishop, 1928). The temporal variations in threshold occurring under these circumstances

[1] This investigation was aided by a grant from the Rockefeller Foundation to Washington University for research in science.

[2] This subject has been fully discussed recently by Adrian (1932) and by Hoagland (1935).

can be traced with a fair degree of precision by the shock-test method. At the cathode of a subrheobasic current the threshold falls rapidly to an effectively steady level, the "plateau," and this is terminated after 1 to 2 milliseconds (msec.) by the onset of this cathodal depression (Erlanger and Blair, 1931b). The latter develops relatively rapidly at first and then more and more slowly with time. Individual axons, we have found (Blair and Erlanger, in press), behave in this respect just as does the whole nerve; though, owing to a spontaneous fluctuation in a fiber's excitability, equivalent to a change of about 10 per cent in the stimulating voltage, the abrupt termination of the plateau is more difficult of demonstration than when large numbers of fibers are contributing to the response. Increasing the strength of the rectangular current beyond the threshold ordinarily does not make for repetition, because, after the fiber has responded to the make, cathodal depression nevertheless develops and quickly carries the excitability of the fiber below the firing-off level despite the high applied voltage. A curve illustrating this phenomenon has been published in another place (Blair and Erlanger, in press).

However, an excised frog's nerve, as is well known, may respond to a constant current with a long continued discharge (Pflüger's tetanus). According to Ebbecke (1924) a repetitive response to constant current stimulation is the normal reaction of human nerves *in situ*. The best account we know of dealing with the conditions that make for repetition in response to a constant current is given by Gotch (1900, pp. 509–12). "The opening tetanus," he says in part, ". . . . is due to a state of prolonged excitation at the anode. . . . . The effect is increased if a current is closed in the reverse direction, thus making what was formerly the anode into a cathode." His discussion of the subject is closed with the indefinite statement that "there are . . . . clear indications that in some nerves there are prolonged excitation phenomena, even at closure."

Such a record should perhaps have suggested to those who in recent years have dealt with the subject of repetition in nerve fibers the desirability of considering the "anelectrotonic state" as a factor contributing to the development of the repetitive type of response, the more so in view of the belief, now so widely expressed (Monnier and Jasper, 1932), that undisturbed nerve acts as if it were anodally polarized (subordinated (Lapicque, 1923)). It was not, however, until we had been investigating the subject of induced repetition for almost two years without having succeeded in finding a satisfactory interpretation of the varied results,[3] that we were led by them to ascertain whether we could not through the use of frank anodal polarization bring some reason into the problem of repetition.

INDUCED REPETITION: METHODS. The amplifying and recording meth-

[3] Some of the results were reported at the joint session of the American Physiological Society March 31, 1934.

ods have, in general, been those described in our previous papers dealing with the responses of individual axons (Blair and Erlanger, 1933). The nerve employed for the most part has been the phalangeal preparation of *Rana pipiens*. When in full length it usually has been mounted on eight electrodes. The attenuated peripheral end of the nerve rested on the grid (most distal) lead, the ground lead, also designated electrode 1, and on electrodes 2 and 3. Over these the nerve ran a straight vertical course so that it could be moistened by a dripper. It then was draped horizontally over electrodes 4, 5, 6 and 7. The distances between electrodes were not always the same; but usually they were about as follows: between grid and ground (or 1), 1 and 2, and 2 and 3, 8 mm. each; between 4 and 5, 5 and 6, 6 and 7, 20 mm. each. The length of nerve subtended by the successive electrodes was about the same as these electrode separations excepting between 3 and 4. Any slack it was necessary to take up in order to place

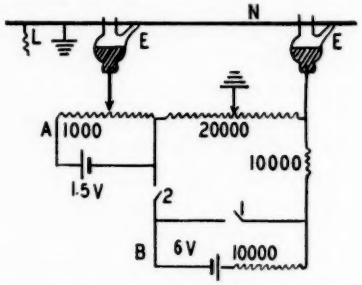

Fig. 1. Diagram of circuit employed in imposing on the nerve, *N*, the continuous, *A*, and the rectangular, *B*, potentials. The testing circuit for delivering induction shocks or condenser charges, has been omitted.

the central end of the preparation on electrodes 6 and 7 usually was collected between electrodes 3 and 4; since the preparations sometimes were as long as 140 mm., the span here was wide and much more varied than elsewhere. Currents could be applied to the nerve through any desired combination of electrodes 1 to 7.

When the nerve was to be continuously polarized anodally two constant current circuits were employed, one (*A*, fig. 1) for this purpose, the other (*B*) to supply the "rectangular" stimulating current. The "continuous" current operated through the potential divider, the bridge balance and the nerve's resistance. The latter at times was perhaps as high as 500,000 ohms when the nerve was unpolarized; but the nerve resistance probably increased as the current continued to flow and, as we had no means of measuring the current flow in this circuit, the anodal effects could not be correlated with current flow; neither could this be done when the two currents were used simultaneously. The rectangular current could be given

the desired strength while the battery A was in or out of circuit; depending upon the end in view, the former was set just below the rheobase or at any desired level above it. The rectangular current was made by the break of a short-circuiting switch and broken by the opening of a series switch. That it was steady is evidenced by the many experiments in which a common electrode has served as both the cathode of the polarizing current and the ground lead into the amplifier: under these conditions, with the circuit unbalanced, the record is perfectly flat, when a variation of a few microvolts in the applied potential would have been recognizable in the picture on the face of the tube. The rectangular currents were usually applied for 0.2 to 0.3 second about once a second. All electrodes were thoroughly satisfactory calomel half-cells. The Ringer's solution used was quite innocuous; nerves refrigerated in it have remained irritable for as long as five weeks (Peugnet, unpublished).

It can be seen in figure 1 that in the course of a cycle of our rotating interrupter the current through the nerve in experiments in which circuit A was closed, changed slightly when switch *2* closed; and that when switch *1* opened the nerve had added to it the full "rectangular" potential. Whether the latter actually reversed the current flow through the nerve we do not know, but we doubt if it did. In order to assure ourselves that these complications did not qualify our results, in one experiment the two circuits were arranged in tandem on the nerve with one electrode serving in common as the anode of A and as the cathode of B. By these means early repetition induced by B could be converted into late repetition by the addition of A, just as with the arrangements usually employed (see below).

RESULTS. *Preliminary description of repetition induced by the rectangular current.* Repetition induced by the rectangular current is characterized, as will become obvious, by its unpredictability. In general, repetition occurs more commonly when the region polarized is the thick, in contradistinction to the thin, end of the nerve, but, as a matter of fact, the majority of the preparations contain no fibers that will repeat. Of those that do contain repeating fibers the number thus responding may vary from 1 up, and the number repeating at the various electrodes also may vary unpredictably. Occasionally the same fiber will repeat no matter where the rectangular current is applied, but usually only when it is applied at more or less definite loci. If repetition can be induced at all by the rectangular current alone, it is in evidence from the first. Usually it then gradually fails and, after a while, can no longer be elicited. There have, however, been a few preparations which have yielded repetitive responses over a period of two or even three days, with overnight interruptions during which the nerves were refrigerated. The repetition thresholds in general are lower the larger the fiber. It is for this reason that our observations have been confined exclusively to the responses of relatively large fibers.

Repetition may be either rapid or, in our experience, very rarely, slow.

The rapid repetition, at least, seems to follow two general patterns with many variations and, perhaps, transitions. The most common variety is a series of repetitive responses of which the make response is the first ("immediate repetition"). A current of rheobasic strength may suffice to elicit a short series; then the duration of the repetition can be increased by increasing the strength of the polarizing current. The number of spikes usually does not exceed 8 to 10, though there have been some very much longer trains. Further increase in strength then shortens the series from behind. Usually, though, the threshold of immediate repetition is well above the rheobasic level. If a strength of three or four rheobases fails to reach it further increase as a rule is without avail. This type of repetition usually fails quite rapidly with time, but even after it is no longer obtainable the make threshold may still be as low as ever.

The other type of rapid repetition is characterized by a delay in its initiation ("delayed repetition"). The interval between the closing of the current and the beginning of the repetition is longer, it may be, very much longer, than the sum of the utilization period and the conduction time involved. Thus a delay as long as 0.2 second has been observed, though no effort has been made to determine the limit. The delay varies considerably from trial to trial under conditions which seemingly remain constant.

The usual delays, however, have been of the order of 20 to 80 msec. Extremely rarely the repetition delay has shifted irregularly in successive trials from 0 up to 20 or 30 msec., or more. Increasing the strength of the rectangular current shortens the delay but does not eliminate it; at the same time it increases the length of the burst; eventually, however, it shortens it from behind until it is wiped out completely.

Very rarely one and the same fiber has appeared to be yielding both an immediate repetitive response and a delayed repetitive response during one and the same current application (see fig. 4, D). Of this one cannot, however, be absolutely certain, since there is always the possibility that preparations behaving in this manner contain two axons yielding spikes of the same height and degree of diphasicity, one repeating immediately, the other after a delay. Preparations that contain a number of fibers that repeat late, as a rule, perhaps, also contain some that can be made to repeat immediately (see fig. 4). These different response patterns may be combined in every conceivable way even when elicited through one and the same point of one and the same preparation, some axons responding after each of the fashions. The same fiber activated at different points along the preparation may respond with different patterns, though usually with the same pattern at any given point. Again, there rarely have been fibers that have repeated according to the same pattern at all points.

Usually the repetition pattern obtained from a fiber at a given point remains more or less true to type even if and as repetition gradually fails.

Occasionally, though, the pattern has changed suddenly and radically. A very striking and unusual example will serve to illustrate what can happen. One of the fibers of a preparation yielded immediate repetition at all points. In other fibers delayed repetition waxed and waned with the time at the several electrodes as indicated in figure 2. At a time when delayed repetition had almost completely disappeared (just before the 19th determination) a drop of Ringer's solution falling over the attenuated vertical portion of the nerve, abruptly and markedly increased the delayed repetition that could be induced by stimulation at several remote points on the nerve quite out of reach of the drop (through electrodes 4, 5, 6 and 7). That this was not just a coincidence is indicated by the fact that the same

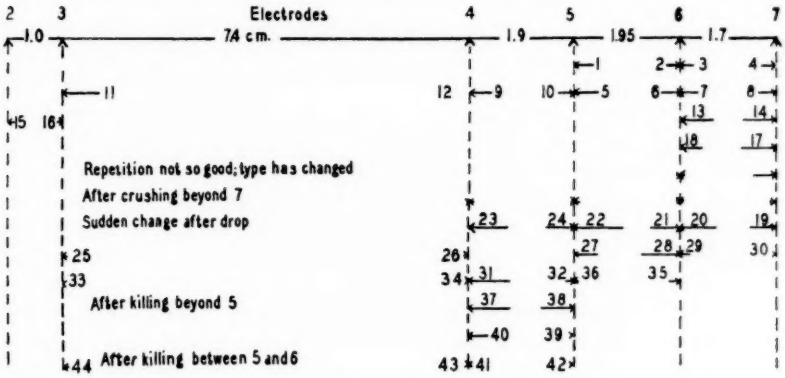

Fig. 2. 5/21/35. Diagram illustrating the behavior of the most capricious nerve by far of the series.

In the top row are the electrode numbers, in the second row, the length of nerve in centimeters subtended by adjacent electrodes. Each arrow indicates by its length the relative activity of repetition when the electrode it points toward was cathode and the neighboring electrode it points away from, anode. The numbers on the arrows indicate the sequence of the determinations.

phenomenon was observed a second time during the course of the experiment. It should, perhaps, be added that the alterations produced by the drop are not attributable to a redistribution of the applied potentials made possible through an alteration in the balance of the bridge-balancing device. For it has not been possible to modify repetition by manipulation of the bridge balance.

These peculiar distributions and sudden variations of the nerve's responses suggested the thought that demarcation potentials and their variations might be responsible for repetition of the delayed type, at least, and for its variability. The same thought was suggested also by the comparative excitabilities of a fiber at the two electrodes when from one the

rectangular current elicited only delayed repetition and from the other only an initial response or immediate repetition. In the majority of such cases, but not in all, the excitability at the former was considerably the lower; the ratios of the excitabilities, as measured either by the make of the constant current or by an induction shock, often was as small as 1:3. It was not possible to account for these differences in excitability as being due to differences in electrode size or in nerve size. Since, however, they could be the expression of local anodal polarization and since local demarcation currents might anodally polarize nerve fibers locally it was decided to determine whether, and if so how, anodal polarization affects the repetitive responses induced at the cathode of a rectangular current.

Effect of anodal polarization on the gross manifestations of repetition. The results obtained demonstrated conclusively that anodal polarization plays an important rôle both in maintaining repetition and in determining its character. In support of this statement the significant data from two experiments may be cited. But in weighing this evidence it is necessary to bear in mind that we have been able neither to measure the strength of the continuous current nor to control any effects the duration of its action may entail. It is also necessary to bear in mind that for each repeating fiber there is an optimum rectangular voltage, a voltage that elicits the longest series of spikes, and that this factor, likewise, has not yet been satisfactorily controlled quantitatively.

Case I (5/30/35, fig. 3). The preparation contained only one, a large, fiber capable of responding repetitively to the rectangular current. For this reason it will be used repeatedly for purposes of illustration. The currents were applied through electrodes 4 and 7. The rectangular current, applied alone with cathode at 4, induced immediate repetition (*A*). The continuous current with anode at 4 converted this, in time, into delayed repetition (*B*, *C*, *D*, *E*), the delay finally growing to 52 msec. The last four spikes, combined, of all five repetitions each subtends almost exactly the same time span. Taking into consideration the fact that repetition was on the wane as the experiment progressed, the increase in the number of responses in *C* may be regarded as evidence that the anodal polarization has, if anything, improved repeatability. This effect, though, has been indicated more clearly in other experiments.

Case II (6/19/35, fig. 4).[4] With the cathode of the rectangular current at either 6 or 7 only initial repetition could be obtained, but a number of fibers responded in that manner. In record *A* the spikes of the fiber that repeats best can be seen repeating in the clear. The repetition intervals are 3.5 and 3.2 msec. With the cathode of the rectangular current and the anode of the continuous current at electrode 6, records *B* and *C* were

[4] Nerve 24 hours old; on the first day the repetition had been of the slow type.

obtained. In both there is a delayed repetitive burst beginning, in *B*, 29 msec. after the start of the rectangular current, and in *C*, 31 msec. thereafter. The duration of the last two repetitive intervals ranges between 3.3 and 4.0 msec. If it be assumed that the fiber repeating in the clear in

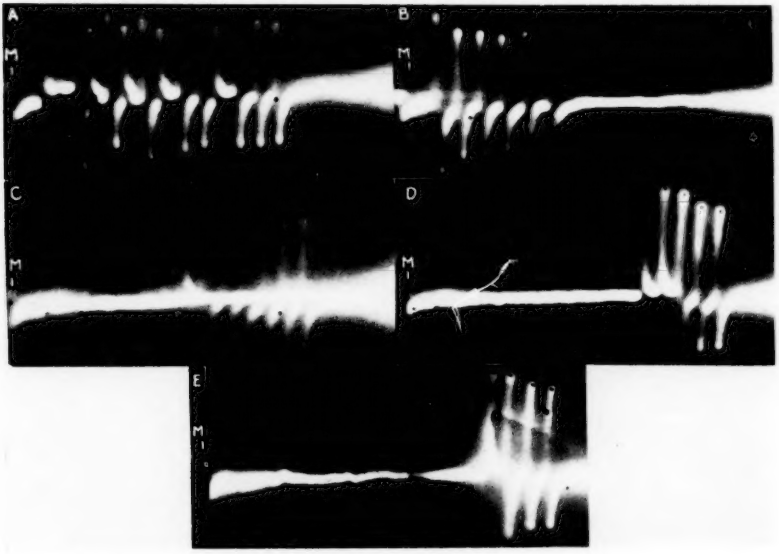

Fig. 3. 5 30 35. Effect of anodal polarization on repetitive response. During the experiment the ability of the fiber to repeat diminished steadily. The action potential does not always reach the distal lead; it then records monophasically. Note that it gets to the distal lead more frequently as the record runs. *M* is the make of rectangular current.

A, rectangular current alone; *B*, *C*, *D* and *E*, continuous (anodal) current added. It is believed the strength of the rectangular current was increased between *B* and *C*.

Below are given in milliseconds the make-response intervals (first number) and the successive spike, or rather crest, intervals. Because of the method of measurement the latter values are affected slightly according as the spikes are monophasic or diphasic.

A. 5.0, 3.2, 2.9, 2.6, 3.1, 3.0, 3.2, 3.6, 3.3, 3.9, 3.9, 3.5, 4.4, 3.9.
B. 6.3, 3.5, 4.2, 3.7, 4.0, 5.2.
C. 31.3, 4.0, 3.5, 4.0, 3.6, 4.1, 3.8.
D. 44.1, 3.2, 4.0, 3.4, 3.8.
E. 52.3, 3.1, 4.8, 3.5.

A has been repeating from the start the immediate burst might have consisted of 8 spikes, at the most. In *B* and *C* the delayed bursts consist of 10 and 11 spikes, respectively. If the additional assumption be made that we are dealing with the response of one and the same axon, and it would

appear that we are, it follows that the anodal polarization has converted the immediate into delayed repetition and increased somewhat the number of the responses. In addition there are one or two earlier, much more widely spaced responses (slow repetition?), probably not of the axon that

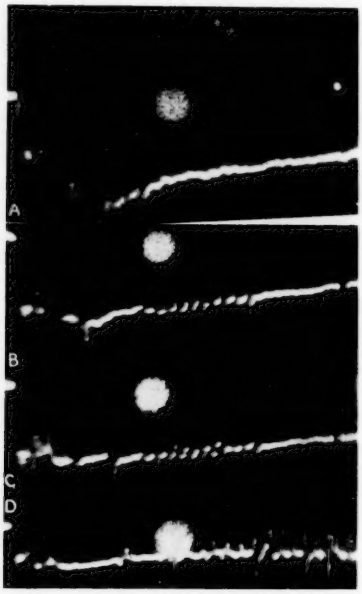

Fig. 4. 6 19 35. Effect of anodal polarization on repetitive response. The make of the rectangular current depresses the base line.

A. Rectangular current alone, cathode at 6. Immediately a number of fibers respond repetitively. The axon seen repeating in the clear probably is repeating from the beginning. Visible spike intervals 3.5 and 3.2 msec.

B and *C*. Continuous current added, anode at 6.

B. Delay of repetitive burst, 29.0 msec.; spike intervals,—3.0, 3.5, 2.8, 2.7, 3.0, 3.3, 2.7, 4.0, 3.3 msec.

C. Repetitive burst delay, 31.0 msec.; spike intervals,—3.0, 2.3, 3.0, 2.7, 3.3, 3.0, 2.7, 2.7, 3.6, 3.7 msec. Some slow repetition in another axon.

D. Rectangular current strength increased. Larger fibers stop responding, smaller fibers respond with immediate and delayed repetition.

yields the later burst. Combinations through electrodes 5 and 6 yielded results comparable to these.

It might be added here that when the strength of the rectangular current was increased (*D*) the large fibers ceased responding and two smaller fibers responded, both with delayed repetition, and one of them, possibly, with immediate repetition also (see footnote 5). The delay, in accordance with the almost universal rule, was briefer (0.042 as compared with 0.065 msec.)

the smaller the axon repeating under the same conditions. Fibers that will not repeat under stimulation by the rectangular current alone can, at times, be made to respond in that manner by carrying out the experiment in anodally polarized nerve. And, at times, fibers that yield only a few spikes, can by this treatment be made to give very much longer repetitive bursts.

Slow repetition. The repetition thus far considered, both immediate and delayed, has been rapid. With the exception of but two cases it has been the only kind of repetition we have succeeded in inducing. The two exceptional repetitions were derived from the two nerves of one frog. The repetition pictures obtained closely resemble those appearing in optic fibers, for example, in response to illumination of the retina: the repetition was slow and irregular. The significant observations on one of the nerves will be described (6/18/35, fig. 5). The currents were first applied through electrodes 6 and 7. Delayed repetition was not obtainable when the cathode of the rectangular current was at 7, but when this current was added through the continuous current with anode at 7, delayed repetition appeared in addition to early repetition. The repetition was of the slow variety, of the kind illustrated in figure 5. This figure, however, reproduces records obtained by the application of the rectangular current only and through electrodes 5 and 6 with cathode at 5. In other words, at one locus the fiber responded to the rectangular current alone with delayed repetition; whereas to obtain delayed repetition from another locus it was necessary to polarize it anodally in addition. In figure 5 the succession of the records is the order of increase in the strength of the rectangular current as indicated by the height of the make artifacts (*M*).

There are good reasons for believing that in *A*, *B*, *C* and *E* only one axon is responding, that this is also the axon giving the large spikes in *G* and *H*, and that the smaller spikes are all from another axon, possibly from two axons (see legend). In *B*, *C* and *E* the large fiber responds with immediate repetition of the quick type, or something akin to it, consisting of two spikes, and this is succeeded by slow repetition (not delayed). For *F* and *G* the current strength was about four times that used in *A*, but the immediate responses have disappeared,[5] and the repetition is now definitely of

[5] This phenomenon, seen also in figure 4, requires a word of explanation. Delayed repetition usually is not preceded by an initial response and this, as will be seen, is consistent with the concomitant curve of thresholds. But when the delayed repetition is preceded by an initial response the curve must have the configuration of *C* (fig. 7). Now, in the latter case, increasing the strength of the rectangular current could eliminate the initial response only 1, if it blocked the fiber (and it is hard to believe that this could be happening here because the current is descending) or 2 if the post-cathodal depression left by the break of the rectangular current lasted until this current is made again something less than a second later. In the absence of an opportunity to put the question to the test of experiment we are inclined to attribute the anomalous behavior to the latter of the alternatives.

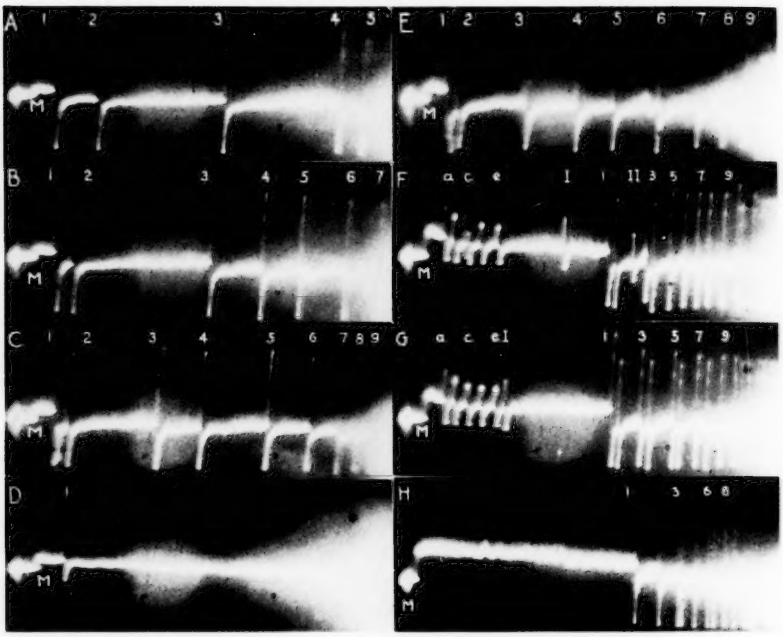

Fig. 5. 6-18-35. Slow (and fast) repetition. Rectangular current only, cathode at 5, anode at 6. Changes in current strength are indicated by the height of the make artifact, *M*. Time falls off logarithmically from left to right. Certainly two, and probably three, fibers (I, a and I) contribute. Intervals in milliseconds are as follows:

A. Make to 1, 5.1; 1–2, 16.4; 2–3, 62.0; 3–4, 111; 4–5, 41.

B. Make to 1, 5.1; 1–2, 6.1; 2–3, 62.9; 3–4, 34.7; 4–5, 33.6; 5–6, 58.2; 6–7, 68.0.

C. Make to 1, 5.1; 1–2, 5.4; 2–3, 36.7; 3–4, 22.4; 4–5, 45.0; 5–6, 35.2; 6–7, 44.8; 7–8, 26.0.

D. Make to I, 8.3.

E. Make to 1, 5.1; 1–2, 4.5; 2–3, 24.6; 3–4, 24.0; 4–5, 21.8; 5–6, 30.3; 6–7, 38.5; 7–8, 27.5; 8–9, 36.3.

F. Make to a, 5.1; a–b, 3.2; b–c(?) 4.5; c–d(?) 5.5; d–e, 6.1.

Make to I, 54.8; I–II, 42.3.

Make to 1, 78.8; 1–2, 3.4; 2–3, 23.5; 3–4, 5.7; 4–5, 12.8; 5–6, 14.5; 6–7, 11.2; 7–8, 13.4; 8–9, 14.1; 9–10, 23.3.

G. Make to a, 5.1; a–b, 3.2; b–c, 4.5; c–d, 5.5; d–e, 6.1.

Make to I, 27.2.

Make to 1, 81.0; 1–2, 4.9; 2–3, 16.7; 3–4, 6.3; 4–5, 19.3; 5–6, 6.4; 6–7, 15.3; 7–8, 9.4; 8–9, 18.1; 9–10, 17.3, etc.

H. Make to 1, 157; 1–2, 27.4; 2–3, 26.5; 3–4, 4.5; 4–5, 18.7, etc.

the delayed type, but, for the most part, at least, still slow. The responses of the small fiber follow essentially the same course as that taken by those of the large fiber, but at higher current levels: in *F* and *G* it repeats immediately, but in a fast tempo, (with two widely spaced responses possibly of another fiber in *F*) but stops repeating in *H* with further increase in current strength.

On account of the limited opportunity we have had to study slow repetition (two preparations only) we have succeeded in acquiring relatively little information in regard to it. Practically the only observation of any importance relative to our thesis is that it is subject to the same control by anodal polarization as is fast repetition. One may also be justified in inferring from the behavior of the fibers in experiment 6/18/35 (fig. 5) that the conditions determining fast repetition and slow repetition, and the conditions determining early and late repetition are not very widely different, otherwise such sudden shifts in the repetition pictures as are seen here would be impossible. Of this we shall have more to say later.

Excitability studies. A. *In nerves not continuously polarized.* Among the conditions necessary for repetition to manifest itself one would expect to find the continuance of a low threshold through the period of the repetition. In our past studies of axons we (Erlanger and Blair, 1931b) have occasionally encountered instances, then regarded as anomalous, in which the threshold under a subrheobasic current, after having typically risen from the low level of the initial "plateau" has exhibited another, and much more deliberate, fall and rise. This and other variations from the rule, including failure of the threshold to rise at all from the level of the plateau, have frequently been encountered in the present studies, and it has been possible to demonstrate a fair correlation between the behavior of the threshold and the types of repetition. A couple of instances dealing with delayed repetition may first be cited.

Case 1. 4/9/34. In many of the experiments in which the changes in threshold in axons capable of responding with delayed repetition have been mapped at the polarizing cathode a result has been obtained comparable to that illustrated in figure 6. Here the threshold, plotted in terms of primary milliamperes necessary to excite, after having risen from the low level determined by the make of the subrheobasic current (the plateau,—points 3 and 4) fell, again temporarily, to a lower level even (points 13 and 14) than had been reached during the plateau stage. The excitability maximum (threshold minimum) was reached in about 30 msec., which was well within the range of the repetition delay. Axons yielding this type of curve from the locus of the cathode of the rectangular current usually are quite unstable in the sense that the threshold level and the configuration of the derived excitability curves are apt to change from moment to moment (see legend).

To plot curves, such as the one just described, requires considerable time. With these quickly changing nerves it is necessary to derive information quickly. One can obtain a general idea of the way the threshold varies with the time by noting in which direction one must vary the strength of the testing current to keep it at threshold as its artifact is moved across the screen through the artifact of the rectangular current; one can even read the strength of the testing current at selected positions (times) and thus quickly

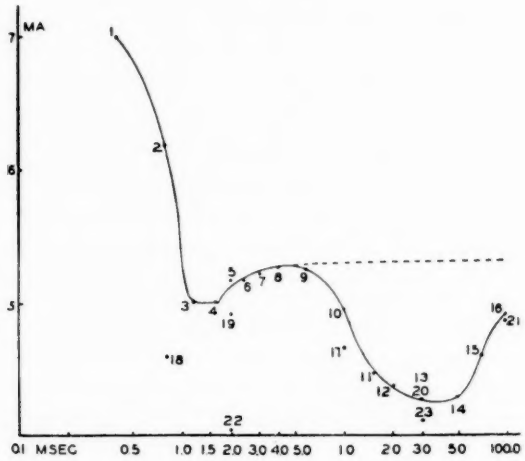

Fig. 6. 4/9/35. Threshold pattern at the cathode of a rectangular current. At the beginning of the experiment the current was set at 80 per cent of the rheobase but at the end the current strength was 88 per cent of the rheobase. The fibers previously had repeated with a delay ranging between 25 and 72 msec. under a rectangular current 124 per cent of the rheobase.

Ordinates, milliamps. in primary circuit (linear); abscissae, current duration in milliseconds (logarithmic). By observing the sequence of the determinations (indicated by the numbers) it can be seen that the curve has the usual typical form through the first 6.0 msec. The threshold then falls again to reach the low point between 30.0 and 50.0 msec. instead of following the normal course indicated by the dotted extension. The instability of these nerves is indicated by the scattering of the points made last.

obtain quantitative data to fit into the qualitatively traced excitability curves.

In case 2 (1/22/35) the analyses were made essentially in that way. The rheobase of the axon happened to be the threshold for the delayed repetition also. The repetition delay ranged rather widely around 80 msec. (16.2°C.). The rectangular current was set at 72 per cent of the rheobase and the excitability of the axon was determined at points 9 and

100 msec. after the make. In the typical excitability curve of past experience the thresholds at both of these points would have been higher than during the initial plateau stage, and at 100 msec. higher than at 9 msec. Here the reverse was the case: the threshold primary currents at the two selected times were 1.87 and 0.92 milliamps., respectively; that is to say, the threshold in the interval between 9 and 100 msec. had just about halved. It will be noted that the correlation between the delay in the initiation of the repetition and the temporal position of this second elevation in excitability is quite good. In many of the cases (some will be described below) the curve of thresholds is without an initial steep decline; it then falls gradually to attain a delayed minimum.

The excitability at the cathode in axons that repeat after a delay does not invariably behave as in these illustrative cases; exceptions, though, have been rare. There has been one instance, at least, of such repetition in which a delayed fall in threshold was not demonstrable. We have also encountered one or two axons that have exhibited this delayed fall in threshold but which did not repeat late.

Excitability studies. B. *In nerves continuously polarized anodally.* The correlation can be greatly improved by stimulating the axon with the rectangular current through a region made continuously anelectrotonic; by these means not only can immediate repetition be converted into delayed repetition, as has been said, but also a delayed fall in threshold can be made to evolve from an excitability curve of the usual configuration. Examples of results obtained may again be drawn from the nerve of 5/30/35 (fig. 3) (see above). The exciting rectangular current was made just subrheobasic in strength and was applied, as while obtaining *A* of figure 3, through electrodes 4 and 7 (separation ca 60 mm.) with the cathode at 4 (fig. 7, *A*). The configuration of the excitability curve was determined by the qualitative method by condenser charges (testing shocks) led into the nerve through electrodes 4 and 5 (separation 20 mm.); but isolated quantitative determinations of the threshold were made about 30 msec. after closing the rectangular current, and occasionally at other times. When the common electrode of the rectangular current and the testing shock was cathode for both, the curve determined in the absence of the continuous current had the configuration (fig. 7, *A*) usually yielded by axons; it fell quickly to the end of the plateau, where the threshold, in terms of the relative voltage necessary to charge the condenser to the threshold level, was 10.4, and then rose somewhat as depression developed so that at 30 msec. the threshold was higher (12.2). Then, without changing any other of the conditions, the continuous current was added with anode at *4* and cathode at *7* and the threshold traced again at *4* (cathode of shock at *4*). The curve (*B*) now fell slowly through 30 to 40 msec. to a minimum of 10.3, or approximately to the value attained during the plateau of *A*. Later in the

experiment, in another trial, without having knowingly changed the conditions, the curve *C* was traced. Here the delayed fall in threshold is still in evidence but there is to be seen in addition the initial decline to the plateau that is characteristic of the excitability curves derived from the cathode of a rectangular current in nerve believed not to be polarized anodally. Obviously, the nerve responding to this test either was not so strongly polarized anodally as it had been, or the make of the rectangular current for some other reason was making itself more strongly felt. It is worth noting that the lowest threshold attained during the delayed fall in *C* is lower even than in *B*.

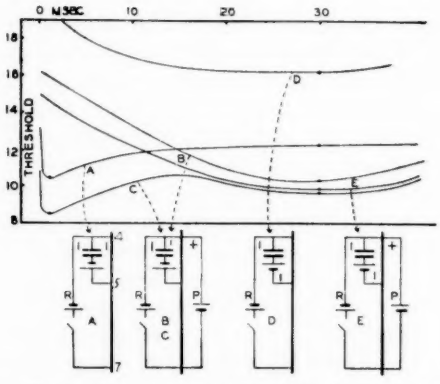

Fig. 7. 5/30/35. Threshold curves, qualitatively traced merely to show their general configurations, with quantitative voltage readings indicated by dots. Ordinates, relative threshold voltage; abscissae, time in milliseconds (estimated except at 30 msec.). The plateaus of A and C are in the positions of the first dots.

Below, simplified diagrams of circuits employed in deriving the data on which the curves, correspondingly lettered, are based.

R = rectangular current; *P* = continuous current. The heavy line is the nerve.

Since in the case of a nerve not subjected to artificial anodal polarization the excitability curve of a fiber that does not exhibit delayed repetition practically invariably has a configuration resembling that of *A*, whereas the excitability curve of a fiber that does repeat, and after a delay, has the configuration either of *B* or of *C*; and since through local anodal polarization of a nerve configuration *A* can be converted into configuration *B* or *C* and at the same time immediate repetition into delayed repetition, it may be concluded that fibers that have exhibited delayed repetition but which were not being subjected to artificial anodal polarization, either were anodally polarized, presumably by local demarcation currents, or else were subject to some other condition which produces a state similar to the anelectrotonic state.

Attention should be directed to the interesting fact, illustrated by this same preparation, that the threshold at the cathode of a rectangular current can attain, though slowly, about the same level in nerve thoroughly polarized anodally as it does, quickly, in the same nerve not anodally polarized. The relevant values in this case were 10.3 and 10.4, respectively (see fig. 7, *B* and *A*). Moreover, it is interesting to note that at the time the latter is in a state of cathodal depression (threshold 12.2 after 30 msec.) the former is at the maximum of its excitability (threshold 10.3), from which it then declines slowly. These relationships, however, undoubtedly will be found to depend upon time, strength and other factors that have not yet been controlled quantitatively. But this exhibit does indicate that a rectangular current of a given strength can develop the same excitability in a fiber that is anodally polarized as in one not previously so polarized.

Is there a characteristic excitability curve for immediate repetition? One would expect the curve of excitability characteristic of immediate repetition to be one in which, to mention extremes, the threshold either falls quickly to a maintained level, or, after having so fallen, then rises slowly along a curve so shaped that it could account for the spacing of a repetitive burst (see section on Repetition Picture).

In case *A* of experiment 5/30/35 (figs. 3 and 7) (it was one that yielded immediate repetition) the excitability curve under treatment of the nerve with a subrheobasic rectangular current had the usual configuration. But the amount of depression developing in a fiber depends on so many factors, among which is the strength of the rectangular current, that it is precarious to venture an opinion as to comparative magnitudes. The depression developing here in 30 msec., calculated in terms of thresholds, amounted to 17.3 per cent, which is perhaps somewhat less than the usual value for fibers that do not repeat.

When one happens to have a fiber that responds with immediate repetition when the current is superrheobasic one can plot the excitability with the rectangular current set above the rheobase. This method might be expected to give a more adequate picture of the significant changes in threshold than one that does not cause the fiber to respond at all. Stressing now only the particular result obtained in such experiments that is relevant to the present subject, it has been found (see fig. 9) that after recovery of the fiber from the refractory state following the make response the threshold, though oscillating, does not rise materially during a period lasting as long as the readings have been continued, namely, 22 msec. This is exactly the behavior that would make for immediate repetition.

Apparently, therefore, there are circumstances under which cathodal depression does not develop, at least within this period of time, and there are reasons for regarding the anelectrotonic state as one of the conditions tending to have that effect. Delayed repetition unquestionably is refer-

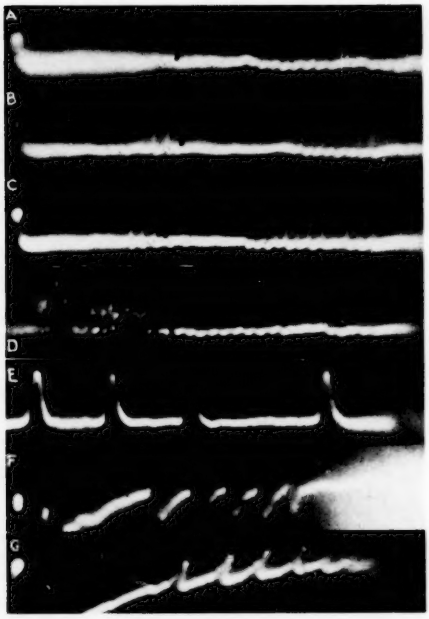

Fig. 8. *A*, *B*, *C*, *D* and *E* are from preparation 4/6/34. The time for them is linear. The stimulus is a subrheobasic rectangular current as may be seen in *A*, *B* and *C*, in which the current, starting in the spot to the left, fails to elicit a make response. The current strength is the same in *A* and *B* and probably in *C*. Of the series of numbers given below the first is the repetition delay, the others the consecutive response intervals, all in msec. The figures in parentheses are recorded response height in millimeters. T = 24°C.

In *A* a single fiber repeats. There is a gap about equal in duration to 3 normal intervals. 92.5 (12.5) 4.26 (11.2) 3.96 (11.3) 4.26 (11.8) 4.57 (12.2) 5.03 (11.9) 4.42 (11.8) 15.4 ÷ 3 = 5.1 (12.4) 4.57 (12) 5.50 (12.)

B. Low series: 53.0 (5.2) 4.88 (4.8). High series: 108.5 (13) 3.96 (11.5) 3.96 (11.4) 4.26 (11.3) 3.96 (11.5) 5.48 (12) 4.57 (11.6).

In *C* the same two fibers are repeating. There is a two-interval gap in the high series. Low series: 43.3 (5.0) 3.77 (4.0) 4.68 (4.5) 5.59 (4.7) 5.59 (4.6). High series: 86.5 (12.8) 3.47 (11.5) 3.92 (11.5) 4.08 (11.3) 4.23 (11.7) 3.77 (11.6) 4.23 (11.7) 4.83 (11.8) 9.5 ÷ 2 = 4.75 (12.3) 4.53 (11.8) 5.28 (11.7).

For *D* the constant current strength was increased slightly but it still was subrheobasic. The beginning of the repetition is not shown. At least three axons are repeating. Besides the two active in *A*, *B* and *C* there is a series from a fiber responding diphasically. There is no tendency toward synchronization of the responses of the different fibers. The long continued series of responses undoubtedly is from the larger axon repeating in *A*, *B* and *C*; it displays a 4-interval and a 2-interval gap.

E. Responses from the large axon responding in *A*, *B* and *C*, taken at a higher speed for accurate measurement of a gap. The successive intervals are 4.7, 4.7, 7.6 ÷ 2 = 3.8; 4.5.

F and *G*. Spikes of a repeating axon recorded at the cathode of superrheobasic

able to anodality. Now, although in frog's nerves a rectangular current does not commonly elicit repetition, it is the rule, when several fibers are repeating, for both types of repetition to be in evidence in the different fibers. It is difficult to believe, if prepolarization is the basis of repetition, that a local demarcation current would polarize oppositely the fibers at a given locus. It seems much more reasonable to suppose that if some of the fibers are repeating late because they are anodally polarized all of the others are repeating because they, too, are anodally polarized. Then there is the case of the fiber in which the repetitive delay in successive pictures fluctuated irregularly between 0 and about 30 msec. Presumably this exhibit is to be attributed to varying degrees of anodal polarization, the result of changing demarcation currents. And we have still another case in which the repetition sometimes was immediate, sometimes delayed, but here the delay times were relatively fixed. The repetition was delayed whenever the rectangular current failed to elicit a make response, and immediate whenever the current was rheobasic. Presumably the nerve's excitability was fluctuating around the rheobasic level and the excitability changes determined by the rectangular current were such that a make response could immediately start repetition (see below) but, failing a make response, repetition would not begin until sufficient time could elapse for the slowly falling threshold, characteristic of anodal polarization, to decline to the discharge level.

We have not, however, through anodal polarization, succeeded in finding a way of converting the usual curve of thresholds into one with a flat floor such as would account best, perhaps, for immediate repetition. Curve *C* of figure 7 reproduces the nearest approach to flatness that has been attained by these means. Such a result, however, holds out the promise of being able to find a set of conditions through which, due to anodal polarization, immediate repetition and a relatively flat excitability can regularly be induced. This type of excitability curve (*C*), seen also in nerve not made anelectrotonic artificially (fig. 6), would account for immediate and delayed repetition in one and the same fiber during one application of the rectangular current. An instance in which this probably obtained (fig. 4, *D*) has been referred to, and there have been others. The same sort of behavior probably accounts for the many instances in which delayed repetition has

currents. The time for these is logarithmic. The sweep starts with the make of the current.

F. 11/21/33. 9.23, 3.77, 3.82, 3.92, 4.02. T. = 24.3°C.

In *G* (11/28/33) the constant current artifact distorts the base line. To save space the record is reproduced with the base line tilted. The irregular spacing of the response arising at the stimulated locus is clearly seen here; 18.1, 6.27, 5.67, 7.22, 6.71, 8.08. T. – 21.9°C.

been induced by a rheobasic rectangular current. Here there is a response to the make and then, after the delay, the repetitive burst.

One not uncommonly finds in response to a subrheobasic rectangular current that the threshold in the interpolar region (and sometimes in the extrapolar region also) relatively close to the cathode falls slowly to a minimum even though at the cathode the threshold, as usual, falls quickly to a minimum and then rises again. This combination is seen in figure 7. For *D* the conditions were exactly the same as for *A* excepting that the testing shock was reversed so that its cathode was in the interpolar region (at *5*) about 20 mm. from the cathode of the rectangular current. The threshold in *D* falls slowly to attain the value of 16.0. When the nerve then was put into an anodal state at *4* (see *E*) and the thresholds determined as before at *5*, they fell along the same sort of curve, but attained a very much lower minimum than in *D*, namely, 9.8. The minimum was not only lower than that of *D*, it was, indeed, as low as the initial low of *A*, and we have no assurance that point *5* was the locus of maximum spatial excitability under the circumstances.

These observations, then, indicate that there are conditions which 1, can render points away from the cathode of the rectangular current more excitable than at the cathode, and 2, can cause the excitability to grow at different rates at different points. It follows that the excitability as measured by a shock might be a record not of the excitability changes occurring at the cathode of the shock (and of the rectangular current) but of any points within the range of the cathode that become sufficiently excitable to dominate points nearer to the cathode, so that as the process of polarization progresses, the dominating point may shift its position.

Such considerations naturally raise the question, *where does repetition start?* Immediate repetition undoubtedly starts at, or at least very close to, the cathode of the rectangular current. This follows from the fact that there never is any interval, greater than the regular spacing, between the first, the make, response and the rest of the burst. And when the record is taken directly from the cathode of the rectangular current the burst, when immediate, starts after an interval that is of the order of magnitude of the utilization period.

In the case of delayed repetition this question is not so simply settled. In view of the fact that an action potential upon passing into an injured locus may initiate an after-discharge there (Schaefer and Schmitz, 1933), the question arises, can our delayed repetition be initiated in like manner? 1. If it were, it would always have to be preceded by a single spike recording at the lead an interval after the make of the rectangular current about equal to the utilization period plus conduction time. But delayed repetition usually is not preceded by a response attributable to the make. 2. Again, if it were, an action potential moving into the injured locus from

any point on the nerve should initiate the after-discharge. As a matter of fact it is extremely rare to find a fiber that will respond with delayed repetition to stimulation at all points along its course; usually repetition can be initiated by stimulation of certain loci only.

For the further discussion of this question it becomes necessary to consider our results from the standpoint of stimulation in an electrotonic nerve. The condition we are dealing with, at least in so far as concerns delayed repetition, is, we feel reasonably certain, related to the state of prolonged excitation that develops at the anode with, and subsequently to, the break

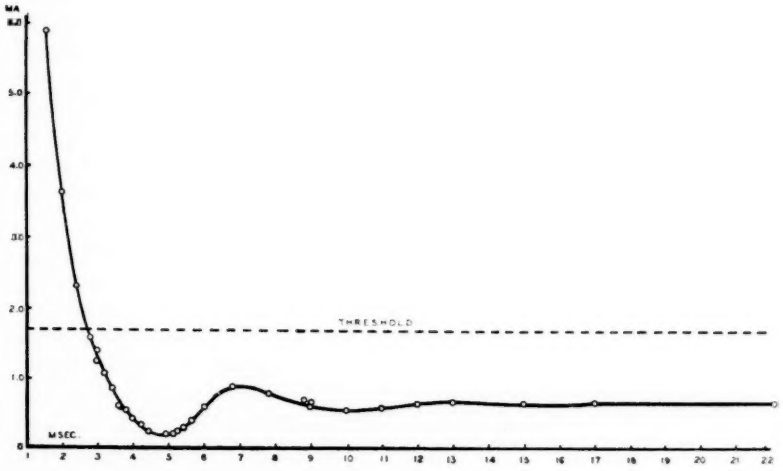

Fig. 9. 12/20/33. Threshold pattern of a fiber at the cathode of a rectangular current of a strength just short of that which elicits a repetitive response (about 3 times rheobase). Ordinates, milliamperes in primary circuit necessary to stimulate. Abscissae, milliseconds with zero at start of rectangular current. The period of the oscillations is about 5 msec. (low thresholds at 5, 10 and 15 msec.). The repetition period also was about 5 msec. The dotted line is the unpolarized threshold.

of a polarizing current. If proof of this is needed over and above that already furnished, it is supplied by the fact that the "opening tetanus," like our delayed repetition "is," to quote from Gotch (1900, p. 511), "delayed in its appearance so as to occur at a distinct interval after the opening twitch." "The causation of the . . . . continued response," Gotch says in another place (p. 512) "is a prolonged molecular change, coincident with the gradual disappearance of an enforced state of greater stability into one of instability." In addition the amount of depression produced by stimulating currents appears to be less in the case of anodally polarized than in normal nerve.

Granting the above premises it follows that when a rectangular current is passed through a fiber that is anodally polarized repetition, or at least delayed repetition, is going to develop at the locus within reach of the transient cathode that is most anodal, even though its threshold, as ordinarily defined, be high. When the anode of the continuous current and the cathode of the rectangular current are at a common electrode, one would expect the repetition to start under that electrode. But when the anodal polarization is the result of fortuitous demarcation currents the repetition, as has been indicated, might start at some point not too far away from the cathode of the rectangular current.

As a matter of fact, we have made some observations that can be interpreted as indicating that delayed repetition sometimes does arise away from the cathode. One of the procedures employed to investigate this question may be described. One electrode is made to serve as the ground lead into the amplifier and as the cathode of the rectangular current. Then, given an axon which responds to the rectangular current with delayed repetition and which exhibits the delayed rise in excitability, one may, while observing the successive trains of responses, gradually shorten the duration of the rectangular current until its break falls in the range of the repetitive responses. Under these circumstances, one spike of the series may occasionally record an appreciable time after the break; the interval has been as long as 0.5 msec. Since there is no "latency" of response to *threshold* constant currents (Blair and Erlanger, in press), it may be concluded that the impulses were originating away from the cathode, as far, perhaps, as 10 mm. It should be made clear, however, that under normal circumstances this can occur no more frequently than there are fibers that repeat after a delay. This, in the frog, constitutes a very small minority of all the fibers we have had under observation.

Some conditions accessory to the initiation of induced repetition. Repetition is induced by the rectangular current when the decrease in threshold it determines permits some rhythmical process to reduce the threshold to the level of the constant current excitation. It is not surprising to find, therefore, that there are a number of ways of initiating repetition when the rectangular current is set below the discharge level.

1. A shock of at least threshold strength with cathode at the cathode of the rectangular current and delivered while the latter is closed is apt to start repetition. *a*. The repetition may then begin immediately, or *b*, not until there has elapsed a period longer certainly than many recovery periods (exact measurements have not been made). In repetition of the delayed type, the shock is most effective when delivered at about the time the repetition would have developed under a stronger rectangular current.

2. Under similar conditions repetition can occasionally be started even when the shock is below the threshold, i.e., when it does not elicit an immediate response.

3. Repetition can be started by an anodal shock which does not elicit an immediate response. In this case a common electrode serves as cathode of the rectangular current and as anode of the shock. The conditions otherwise are as under (2) above. Presumably, it is not the anodal phase of the shock that starts the repetition; indeed, the anodal phase properly placed can, as will be seen, prevent the development of one of a series of repetitive responses. Rather, the impetus must be the rise in excitability that is the invariable sequel of such a shock (Erlanger and Blair, 1931a).

4. Still another procedure that may initiate repetition is the passage of an action potential into the locus that is subject to the action of the cathode of the rectangular current (see also Ebbecke, 1924).

5. Repetition can be started also as a result of the rise in excitability that is responsible for the treppe phenomenon; indeed, treppe may be the explanation of some of the results just enumerated. But in this connection we wish to cite an instance in which an unusually long treppe period undoubtedly was responsible for the initiation of the repetition. The preparation contained several axons which could be made to repeat with a subrheobasic constant current. It so happened that a rectangular current, one lasting about 0.1 second, that was just below the repeater threshold occasionally stimulated one or more axons upon breaking. This current, as usual, was being repeated at intervals of about 1 second. Whenever a break response occurred, the next application of the constant current and several of the succeeding ones resulted in bursts of repetitions appearing while the rectangular current was closed, but some time after its make,—with a long delay, in other words. We have good reasons for believing that in this preparation the increase in excitability resulting from a single response lasted through a second at least; and it is concluded, therefore, that the repetitive bursts occurring during the rectangular current developed in the treppe that was initiated by the response elicited by the break of the constant current.

Experiments on the phrenic nerve. It was deemed desirable to repeat some of the preceding experiments using a nerve that is unbranched and preparable with a minimum of damage. The phrenic (of the dog) was the nerve selected. It was whipped out without making any attempt to get it clean, placed in Locke's solution in a refrigerator for a few hours or longer and then mounted on electrodes arranged about as usual. The phrenic nerve is so thick that even when it is cleanly removed from its sheath the activity of individual axons is at the limit of visibility under the maximum amplification available. The present observations, therefore, deal with the activity not of single, but of many, axons. The nerves were in air at room temperature and uncleaned, except where otherwise stated.

Nerves observed within a few hours after removal exhibit intense spontaneous activity, the like of which is never seen in frogs' nerves (see below). They evidently are in a state that makes for repetition. Indications that

such is the case are given also by the behavior of the nerve in response to stimulation. Whether the stimulus be a single shock or the make of a constant current the response consists of an immediate series of 3, 4 or more elevations decrementing in height. The total response obviously consists of summed spikes of axons discharging repetitively, the decrement in height presumably being the result both of differences in the length of the repetitive chains, and of increasing phase differences between the responses, of the individual contributing axons. The alternative way of accounting for this effect of stimulation, namely, that the shock does not induce repetition but merely brings into temporary superposition the responses of fibers that are repeating spontaneously but out of phase, seems an unlikely one, because the rate of this repetition is relatively very much faster than spontaneous repetition has been in our experience in the frog.[6] It would seem then that we are dealing here with a repetitive response to a single shock. It may be added that although there have been some local differences in the threshold for this type of response, it has been possible to obtain it from any point on a preparation. Frog's nerve in our experience responds repetitively to a shock only when it will repeat on stimulation by a rectangular current, and then the shock must be delivered while the constant current is closed. But then, as has been seen, it will repeat in response to the shock when the constant current itself is below the repetition threshold. Apparently the phrenic nerve will repeat to a shock even in the absence of the constant current. This particular phrenic when treated with a subthreshold rectangular current, yielded from both the cathode and the interpolar regions near the cathode excitability curves of the type seen in figure 7, *C*. Judging by the configuration of the curve one would have expected the nerve to have responded to a sufficiently strong constant current with repetition of the immediate variety.

After having been in the refrigerator over night one phrenic nerve, which previously had behaved as had the above, was no longer repeating spontaneously; and neither would it respond repetitively to shocks. The effect produced by rectangular currents differed at different loci and did not seem to follow any rule; at some points the response was delayed repetition, at others there was only an initial spike. In the former cases the threshold curves fell slowly to a delayed minimum, in the latter they fell quickly to a minimum and then immediately rose. Based on experiments with frogs' nerves this exhibit indicates that there were along the nerve loci that were anodally polarized.

Another preparation (6 hrs. old) responded to the rectangular current with delayed repetition when surrounded by its sheath with its adherent tissue, but with only a single spike when desheathed. Therefore, with still

[6] Adrian (1930), however, has observed fast rates in spontaneously active fibers of the phrenic.

another preparation (one 24 hrs. old), after it had been shown that stimulation with the rectangular current yielded, at all points tested, delayed repetition and threshold curves of the type attaining a delayed minimum, half the length was cleanly desheathed and the nerve was then draped so that the sheathed half was on electrodes 2, 3 and 4, the desheathed half on electrodes 5, 6 and 7. Rectangular currents applied through any combination of 2, 3 and 4 all yielded the same type of response as before, namely, only delayed repetition, but combinations of 5, 6 and 7 gave only initial responses and no delayed repetition. The nerve was then turned so that the desheathed part was on 2, 3 and 4, the sheathed part on 5, 6 and 7. Again the rectangular current elicited from the sheathed part delayed repetition and from the desheathed part only initial responses. Threshold curves from the desheathed part fell quickly to a minimum and rose sharply almost immediately. The cathodal depression was, relatively, by far the most extreme we ever have seen.

It is obvious, therefore, that the sheath, which in the phrenic nerve is quite a massive affair and separated from the nerve proper by loose connective tissue, supplies a factor which makes for repetition when a rectangular current is passed into the nerve through it. This behavior is quite in accord with Adrian's finding that stripping the sheath for 1 cm. from either end of a phrenic preparation abolishes or reduces spontaneous repetition. Adrian argues (1930), though without convincing himself, that the sheath makes for spontaneity by so guiding demarcation currents that the nerve becomes polarized cathodally. Our observations show, however, that the sheathed nerve, at some loci, at least, behaves as does frog's nerve when anodally polarized. Therefore, if the sheath acts by directing current lines through the nerve, it would seem that both spontaneity and induced repetition originate in regions anodally polarized thereby. There are, of course, other possible factors. The sheathed nerve in excised preparations may be acting abnormally due, for example, to lack of O_2, and it will be shown later that this may be a factor. Or, to suggest the other extreme, the desheathed nerve may be the abnormal part due to its exposure to an abnormal milieu.

Adrian, it will be recalled, traced the origin of spontaneous repetition in the phrenic to injured fibers coursing along cut or torn blood vessels. In the light of our observations this finding suggests another way than Adrian's of accounting for the picture of spontaneous repetition he describes. Demarcation potentials will polarize some of the fibers anodally; the musculature of blood vessels probably is spontaneously rhythmical and its contractions might cause currents to flow in such a way as to produce cathodes along the anodally polarized fibers. The conditions then would be exactly those that elicit delayed repetitive bursts.

THE REPETITION PICTURE. As a preliminary to the phases of the problem of repetition thus far considered it has been necessary to know only

that rapid repetition may start either immediately or after a delay. We now propose to consider the factors that determine the architecture of the bursts. The pictures obtained differ somewhat depending upon the presence or absence of conduction from the stimulated locus. The conducted response will be described in detail because it is undistorted by the make transient. Where there are significant differences between the two they will be specified.

Immediate repetition usually consists of a single shorter or longer burst of responses whereas a delayed repetitive response rather more frequently is made up of several bursts. The first spike of any burst practically always is the highest (see, e.g., fig. 8), the second usually the lowest, though the decrease may last through three or four of the responses. The spikes then usually increase to a constant height which is maintained to the end of the burst, the last spike of a group usually being nearly, if not quite, as high as the first. The differences in height, though, may not be particularly striking. Such as there are cannot be referred to a change in the base line resulting from a changing residuum of after-potential, such as is seen when nerve is stimulated by shocks at a constant, rapid rate (Gasser, 1935). There is no appreciable change in the time to maximum of the successive spikes of a group.

In a typical group the first spike interval tends to be the shortest (see fig. 8, and legend) and the intervals between successive spikes then increase, slowly at first, and rapidly as the end approaches, the last interval almost invariably tending to be the longest. The spacing, though, is never perfectly regular, and the irregularity definitely is greater when the record is taken from the site of origin of the spikes, than when conduction is involved (see fig. 8, *F* and *G*). Conduction apparently tends to eliminate some initial irregularity of spacing. On account of this irregularity the rate of increase in spike spacing cannot be estimated with any great degree of accuracy. All that can be said is that in bursts of seven or eight spikes the increase usually is less than 40 per cent. When several axons are repeating, the last spike intervals of each are the only ones that can fairly be compared; for they, presumably, are evoked under just threshold conditions. On this basis one may say that, in general, the spike interval in different fibers is longer the lower the response height of the fiber. The differences, though, are not great. The range probably is as wide as could be expected taking into consideration the range of fibers we are dealing with. The range of spike height is about 3 to 1, giving a conduction rate range of say 25 to 8 m.p.s.; and it is estimated on the basis of previous work (Blair and Erlanger, 1933) that refractory periods in this range increase about 60 per cent. The range of spike intervals might very well be as wide as this on the average (see fig. 8). The repetition delay of small fibers usually is shorter than that of large, but it is difficult

to obtain standard conditions on which to base this comparison. We are not yet in a position where discussion of these relationships would be profitable.

The mean rate of discharge has ranged between about 140 and 370 per second (18–24°C.). This is higher even than Adrian's usual frequencies in the cat at body temperature. The difference, possibly, is due in part to the fact that the two sets of experiments deal with fibers belonging in different excitability ranges, Adrian's being small fibers and ours large, and in part to differences in the conditions determining the repetition; ours is induced by a rectangular current, whereas Adrian's originated spontaneously.

Factors determining the repetition intervals. The picture presented by a repetitive group conveys the impression that it is the result of the initiation of impulses at a rate which at first is considerably faster than the fiber's recovery time but which slows gradually until at the close of a burst the spike intervals are just about equal to the recovery time. For comparison with these intervals we have determined the recovery time in fibers that could be made to repeat. The determinations were made in the usual manner at the cathode of the subrheobasic rectangular current some time after the make. In all cases the relatively refractory periods as defined by Graham (1934) were found to be somewhat shorter than any of the spike intervals and, therefore, considerably shorter than the last interval of a burst. A typical example is the following: the group consisted of four spikes with successive intervals of 5.80, 6.08 and 6.62 msec. The relatively refractory period was 4.0 msec. (T = 18.6°C.). The time to the maximum of any supernormal period there may have been was not determined in these cases. But in another connection, to be considered in a moment, we have made measurements which can be regarded as supplying the equivalent of the supernormal period. It will there be seen that there may be a very close agreement between that period and the repetition interval. Be this as it may, it does not seem possible to account for the group picture on any other basis than as being determined by a frequency of impulses that declines until the interval becomes about equal to the period of rising excitability in the recovering fibers. If the rise in excitability accompanying recovery from refractoriness determines repetition it is natural to expect repetition to fail when the ability to respond has declined to the point where the entire excitability rise available does not suffice to stimulate.

Here it might be added parenthetically that the repetition rate varies somewhat with the strength of the activating rectangular current. To cite one example, the spike intervals with the current at the threshold of repetition (in this case, 1.17 rheobases) were about 2.9 msec.; with a current more than twice as strong (2.5 rheobases) the intervals were 2.4 msec.

The shortening is less than one might be justified in expecting of an increase in excitation as large as this increase in current strength ordinarily produces, but the situation is complicated since cathodal polarization also affects the duration of the refractory period (Bishop and Erlanger, 1926).

Growth of fatigue in the repeating fiber can be ruled out as an important factor determining the group picture because developing fatigue would decrease spike height up to the end, whereas the last spike of a group usually is almost as high as the first. The development of a local block as the cause of the gradual widening of the interspike intervals can be eliminated for many reasons: 1. An impulse initiated by an induction shock between any of the responses of a group is conducted to the lead without undue delay. 2. The record obtained at, or at least very close to, the locus of repetition, it has been seen, is the same as the record after conduction, excepting a slightly greater irregularity of spacing in the former case. 3. In the case of any one fiber all of the group pictures usually are essentially alike; the last intervals all tend to have approximately the same duration; it is scarcely conceivable that the termination of action in all cases is the result of the development of a local block.

The nature of the intermissions (gaps) in a repetitive response. A delayed repetitive response to a rectangular current, it has been said, is apt to consist of several groups of discharges, an immediate repetitive response of but one group. In both cases such grouping of the repetitions as obtains frequently is obviously the result of the loss of just one spike here and there in a series which, but for the missed spike, would have been continuous. If, in such a case, the group spacing departs from two regular spike intervals the difference is in the direction of being a trifle shorter than two (see fig. 8, *D*). Again, gaps between bursts are occasionally seen in the delayed type of repetition which uniformly are a trifle shorter than three of the regular intervals (fig. 8, *A*); and in such cases the departure from three regular intervals usually is a trifle greater than the departure from twice the regular interval when the gap is about equal to two intervals. Gaps of even greater width are seen; whether these, too, are related in aliquot fashion to the basic spacing cannot be so definitely asserted, but in no instance of rapid repetition has mensuration yielded a result that is inconsistent with the aliquot relationship.

In attempting to account for such gaps one naturally thinks first of the possibility of block along the conducting pathway. But all of the evidence indicating that the progressive change in the spacing of the spikes of a group is not referable to a developing block is opposed to this idea. And there is much additional evidence applying directly to the present question that points to the same conclusion. 1. A shock interposed in a gap at any time outside of the absolutely refractory period of the last response will initiate an impulse which will without fail be conducted to the lead. 2.

In some instances it has been possible to eliminate a gap by increasing the strength of the rectangular current. 3. The spikes of the burst following a gap may be more numerous than, and as closely spaced as, those of the preceding burst. 4. When the lead and the cathode of the rectangular current are one and when repetition is of the immediate variety, which assures the initiation of the repetition at the common electrode, there is during the gaps not the slightest sign of an initiation of spikes that fail of conduction. The electrode width is not over 2 mm., and the potential of a spike spreads along nerve from the site of its initiation so as to make itself appreciable at a distance of about 4 mm. (Erlanger and Blair, 1934). The absence during the gaps of any sign of a spike potential, therefore, signifies that the gaps are due not to block, but to failure of a spike to be initiated.[7]

Since local block can, with reasonable assurance, be eliminated as the factor that brings a group to a close, one is compelled, in order to account for the aliquot relation of the gap intervals to the spike intervals, to suppose that the recovery period of a fiber is the first of a continuing series of oscillations in excitability with a period about equal to the recovery period. Some experimental evidence has been acquired indicating that there is such an oscillation.

1. A fiber that requires superrheobasic stimulation to yield immediate repetition is stimulated with a rectangular current that is above the rheobase and close to, but below, the repetition threshold, and the excitability changes are mapped by the shock-test method during and following the recovery from the refractoriness resulting from the make response. A typical result is plotted in figure 9. Following the recovery from absolute refractoriness the excitability of the fiber returns to the steady state through three decrementing oscillations around a steady level,[8] the low threshold points falling at 5, 10 and 15 msec. The first low threshold point must be the equivalent of, if it is not actually, the supernormal phase. Be this as it may, the period of these decrementing oscillations, namely, about 5 msec., was of the same order of magnitude as the period of the repetition of this fiber (see legend). Therefore, even though the amplitude of the final excitability oscillation in experiments of this kind always has been low (possibly because the observations must be made at a current strength that is less than that which causes repetition), we, nevertheless, are inclined to regard their presence as evidence of the tendency of the fiber to continue repeating when, in a gap, the recovery incentive to repeti-

[7] One wonders whether there is any relation between the phenomenon regarded by Barron and Matthews (1935) as intermittent blockage within the central nervous system and intermittent failure of impulse initiation.

[8] This is one of the experiments referred to above illustrating the absence of a rise in threshold in a fiber exhibiting immediate repetition.

tion is lacking. Further support is lent this conclusion by the fact that we have not been able to demonstrate this oscillation in fibers that do not repeat.[9]

2. By means of a subthreshold shock, with anode at the cathode of the rectangular current and selectively placed in a repetition series, one can readily prevent the development of any one of the responses and alter to a limited extent in either direction the temporal position of the subsequent responses of the series without otherwise modifying the repetition picture. However, this result does not necessarily signify that polarized nerve has an inherent rhythm that goes on and maintains repetition when one of the responses to the cyclic process has thus been eliminated, since the period of enhanced excitability which follows the depression induced by a subthreshold anodal shock may be the factor that reinitiates the repetitive series, one element of which has been eliminated by the shock. In an instance in which comparative determinations were made, the maximum of the period of postanodal hyperexcitability followed the anodal shock after about 7 msec. (temp. 14°C.). But the repetition interval itself was 9 to 10 msec.; it approximated the postanodal interval so closely in duration that the possibility has to be entertained, though it seems improbable, that the resumption of the series after the induced gap was determined rather by the subsequent postanodal increase in excitability than by one of a continuous series of oscillations in excitability of the kind that has been found to follow recovery from a response.

SPONTANEOUS REPETITION. The fibers of a freshly prepared phalangeal nerve usually exhibit some spontaneous activity, but, as has been said, they never are as active as those of the phrenic. With but two exceptions in no less than one hundred preparations this spontaneous activity has been confined to fibers whose spikes had an amplitude that was less than a fifth that of the most excitable fiber in the preparation. If the fastest fiber conducts at the rate of 25 m.p.s. we may conclude that the fibers most prone to repeat spontaneously are those that conduct more slowly than 5 m.p.s. In our preparations, examined microscopically, small myelinated fibers are relatively more numerous than large, but not nearly in the ratio of 50:1. Small fibers, therefore, must be more prone to repeat spontaneously than large. In one of the two cases in which the fibers that repeated spontaneously were large the repeating fiber was the largest excitable fiber of the preparation; in the other case, besides the large fiber, there were small fibers repeating also. The ability to repeat spontaneously, therefore, is not confined to the small fibers. The discharges of the spontaneously repeating fibers in all cases have been widely and irregularly spaced.

[9] These oscillations in excitability seem to be distinguished from those described by others (see, e.g., Gasser, 1935) by their periodicity and their brevity.

Repetition induced by treating the nerve with sodium citrate locally through the tubulature of an electrode resembles the spontaneous type in respect both to size of fiber repeating and to spacing of responses. Repetition thus induced does not sum with a rectangular current with the sodium citrate electrode acting as cathode. If the assumption be made that the repetition induced by sodium citrate is effected through local anodal polarization, or something akin to it, this negative result signifies that the sodium citrate renders the locus of its action negative electrically so that the repetition it induces does not originate in the treated locus but in adjacent regions.

Where several fibers are repeating spontaneously there has been no tendency apparent toward synchronization of their responses, such as Adrian has seen in spontaneous repetition in warm-blooded nerve and Hoagland (1933) in fish nerve. It might be added here that neither has there been any tendency toward synchronization of the responses elicited by a rectangular current (see fig. 8, *D*). No matter what the strength of current, such fibers as respond all respond independently of one another. This result confirms our previous observations (Blair and Erlanger, 1932) that the activity of intact fibers of a nerve is not influenced by that in neighboring fibers.

Discussion. It would appear then that in a fiber anodally polarized the state of excitation at the cathode of an applied rectangular potential grows relatively slowly and is not immediately decreased, as in unpolarized nerve, by the intervention of cathodal depression. The resulting relatively steady and high state of excitation puts the fiber in a position to discharge a number of times to intermittent changes in excitability.

The behavior of a fiber responding with repetition to stimulation with a rectangular current conveys the impression that a response is not initiated by the rising excitation until the latter has attained a value that exceeds considerably the fiber's threshold. Then the fiber begins to fire off with intervals which apparently are considerably shorter than the time required for the attainment of maximum excitability following a response. But with entrance into repetition the fiber's general level of excitability seems to fall, and at an accelerating rate, to the point where the recovery of excitability after a response no longer suffices to stimulate, when repetition ceases. Presumably what happens when the response consists of more than one repetitive burst is that in the gaps the general level of excitability mounts again so that the repetitive discharge can be reinitiated by one of the excitability oscillations that continue after the final discharge of a burst. The final cessation of a repetitive response must come when, due to diminished anodality or developing cathodality, the excitation level falls below that needed to permit the oscillating process to attain the fiber's threshold.

This seems a plausible way of accounting for high speed repetition. To

account for slow speed repetition, the kind that occurs characteristically in the normal functioning of nerve fibers, as a response to electrical stimulation it becomes necessary to assume that resting, normal nerve fibers are subject to oscillations in excitability of a rate that can be as slow as the slowest repetition rate. Then when an end organ or the experimenter imposes a potential on a fiber, a fiber that behaves as if it were, or actually is, anodally polarized, it fires off each time a spontaneous oscillation carries the fiber's state of excitation to the threshold level. Reference has already been made to the evidence on record indicating that undisturbed nerve acts as if it were anodally polarized. There is also on record evidence indicating that the excitability of a fiber, to all appearances quiescent, none the less is constantly changing (Blair and Erlanger, in press). These oscillations might have the characteristics needed to produce the normal slow rate of discharge. But it might be necessary to assume, in addition, that the rate of this oscillation increases with the general level of the fiber's state of excitation; otherwise it might not be possible to account for the wide range in rate of discharge exhibited by a fiber under physiological conditions. As a matter of fact it has been possible to show that the range of these spontaneous oscillations in excitability is increased by a subrheobasic rectangular current.

The tenability of the hypothesis, however, is contingent upon finding a means of permitting the slower oscillation rate of the spontaneous process to dominate the faster rate associated with recovery from activity. There seems to be a plausible way through which this could be accomplished. Graham (1934) has shown that in a fiber recovering from a response the excitability rises very much faster when it passes through a supernormal period than when it does not. And, of course, it rises very much higher, also. Supernormal recovery must therefore be much more effective as a stimulus than recovery without a supernormal period. If, then, it be assumed that it is not simple recovery from refractoriness that restimulates but recovery via supernormality, the difficulty is removed. The situation might be stated in this manner: a nerve at the appropriate level of continuous excitation will respond to the slower spontaneous elevations in excitability provided the recovery process following a discharge does not carry excitability to threshold.

There are a number of considerations and observations that fit these views. At the anode of a continuous current not only is nerve in a state in which it is not readily subject to cathodal depression, during which it can therefore repeat while anodality is being reduced through cathodal polarization, but, in addition, its hydrogen ion concentration must be high, and this is the condition that makes for recovery through a supernormal phase (Adrian, 1920). Anodal polarization, therefore, makes for repetition on two scores, and the repetition it induces will be fast if it increases the

hydrogen ion concentration sufficiently. We have already seen that the repetition period matches the supernormal period, but is definitely shorter than the relatively refractory period. Recovery through supernormality is not normal and normal repetition resulting from continuous stimulation is never of the fast variety, at least, at the termination of the response. Matthews has shown that warm-blooded nerve *in situ* discharges at a high rate during anemia (1933); and anemia must tend to drive the reaction of nerve toward acidity. Possibly the great activity of the sheathed phrenic in air, after all, is to be ascribed to anodality plus anoxia with its tendency to increase the concentration of hydrogen ions.

In order to account for many of the vagaries of induced repetition it becomes necessary to assume that damage extending only part way through a nerve trunk can, through the demarcation currents set up, polarize neighboring uninjured fibers. This, at first thought, might be regarded as incompatible with the conclusion (Blair and Erlanger, 1932) that action potentials traveling through some of the fibers of a nerve trunk do not influence the excitability of neighboring inactive fibers. The two situations are not, however, exactly comparable. Thus demarcation potentials can be higher than action potentials. But there is a difference of even greater significance. Action potentials are brief and, therefore, considering their height, relatively ineffective; but there is ample time for demarcation currents to effect the limit of anodal polarization permitted by the potential drop they determine.

Finally, it may not be superfluous, at a time when so much stress is being laid on the humoral mechanism of transferring nerve impulses, to direct attention to the fact that it is possible to duplicate, by the treatment of nerve fibers with an electrical current of the kind sense organs are believed to produce, a type of response that a sense organ normally transmits to its fiber.

It is realized that this investigation raises more problems than it solves. The fault, if there be any, has to be attributed in part to the fortuitousness of the opportunities to study induced repetition and in part to the instability of a fiber that can be made to repeat.

SUMMARY

The repetition occasionally elicited during closure of a rectangular constant current (the so-called closing tetanus) develops in an individual manner in the constituent fibers of a nerve.

In the fibers of a nerve that repeat the repetition may be fast, and then either immediate or delayed, possibly with transitions, or, very rarely, slow.

Plots of the excitability changes at the cathode of a subrheobasic rectangular current in fibers that do not repeat, rise as usual to a plateau and quickly decline as cathodal depression develops; in fibers that repeat with a

delay the curves either rise slowly to attain a delayed maximum or are apparently a combination of the usual and the delayed forms; in fibers that repeat immediately the decline from the plateau may be relatively slight or absent.

Immediate repetition can be converted into delayed repetition, often with an accompanying increase in the length of the burst, and the quick rise in excitability characteristic of the former can be converted into a rise slowly attaining a maximum, by applying the cathode of the rectangular current to the anode of a current running continuously. For this and for other reasons the individual behavior of the fibers of a nerve that respond repetitively to the rectangular current alone, is attributed to local anodal polarization resulting from demarcation currents.

Anelectrotonus, supposed by some to be the state of undisturbed, intact nerve, apparently supplies a condition which permits it to respond repetitively for long periods of time at a temporary cathode.

A fiber that will repeat under a rectangular current may repeat also when that current is below the repetition threshold in response to a threshold or even a subthreshold cathodal shock, to a subthreshold anodal shock, to a transmitted action potential and when the general level of excitation is raised, as, for example, by the treppe phenomenon.

The sheathed phrenic nerve regularly responds repetitively to a rectangular current; the desheathed nerve does not. The former behaves in this respect and in respect to its excitability as does an anodally polarized fiber.

A repetitive response of a fiber may consist of one or more repetitive bursts. The spacing and height of the spikes of a burst indicate that the interspike time at first is shorter than the time to maximum excitability following a response, and that the burst ends when they become equal in duration.

Only those fibers that repeat in a superrheobasic rectangular current recover from the make response of rheobasic stimulation through a series of oscillations in excitability, the crest of the first of which is probably the supernormal phase. The oscillation period is about the same as the repetition period. Since the relatively refractory period is shorter than the repetition period it appears that recovery via supernormality is one of the conditions that make for repetition of the rapid type.

In a repetitive response there often are gaps which seem to result not from blocks but from failures of an uninterrupted rhythmical oscillation in excitability to elicit a response. During such pauses the excitability level of the fiber apparently rises again so that the continuing oscillations can initiate another burst.

It would appear that slow repetition can occur only when the excitability of supernormal recovery does not rise too high. Then, presumably, spontaneous variations in excitability, always demonstrable, determine repetition in fibers whose level of excitation is adequately high.

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THE EXCRETION OF INULIN AND CREATININE AT LOW URINE FLOWS BY THE NORMAL DOG

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It has been found in the normal dog, at moderate and high urine flows, that the simultaneous renal clearances of inulin and exogenous creatinine are identical (Shannon, 1935); and the present work extends the above study of the excretion of these two substances to low rates of urine formation.

CHEMICAL METHODS. The methods used for the determination of inulin and creatinine were the same as those used in the previous study and described more fully by Shannon and Smith (1935).

Recoveries of inulin from plasma at low levels indicate the presence of a blank following absorption on yeast and acid hydrolysis. This was 1.5 to 2.0 mgm. per cent when the dogs were on a crackermeal, sucrose and butter diet, and 2.0 to 3.0 mgm. per cent when maintained on a mixed diet. The amount of this substance in the urine is small, being below 1 mgm. per cent when the urine is diluted to the expected U/P ratio of inulin. The determination of this inulinoid substance in concentrated urine does not yield a valid correction for urine determinations in dilute urine, when the Shaffer-Somogyi method is used, since at low concentrations of sugar there is not strict proportionality between sugar concentration and the equivalent titration. Therefore, in calculating our inulin clearances we have subtracted a blank of 2.0 to 3.0 mgm. per cent from the observed plasma inulin, depending upon the diet, but have used the observed urine inulin concentrations without correction.

Endogenous "creatinine" in plasma and urine was determined on each of the dogs used and this correction was applied to the observed values. This varied from 0.83 to 1.09 mgm. of apparent creatinine per 100 cc. of plasma, and from 0.333 to 0.508 mgm. per minute of apparent creatinine excreted in the urine. The application of these blanks, while raising both the inulin and creatinine clearances, does not cause significant changes in the ratio of the two clearances, the mean of all the ratios being 1.016 before correction and 0.993 after.

Although not true of most specimens of yeast used in this study, some yield, when treated as in adsorption of glucose, a small amount of hydro-

lysable reducing substance, equivalent to 1 to 2 mgm. per cent inulin. Since the urines, before precipitation, were diluted to almost exactly the U/P ratio of inulin, this blank, when present, introduced no error in the determination of this ratio. All chemical determinations were done in duplicate and the mean used in the calculation of clearances.

EXPERIMENTAL PROCEDURE. Normal female dogs weighing from 18 to 22 kgm. were used in all experiments. All water, except that necessary for mixing with the food, was withheld for 48 hours prior to the experiment. The urine flows given here undoubtedly are prevented from reaching lower levels by the presence of creatinine and inulin in the urine, and by the water necessary to dissolve these substances for injection. Inulin, as prepared by Shannon (1935), was given subcutaneously in doses of 1 gram per kilo 45 minutes before the beginning of the first urine collection period. Creatinine was administered subcutaneously in doses of from 100 to 200 mgm. per kilo, 15 minutes after the inulin. This technique yielded blood concentrations of these substances that were fairly constant throughout the two hours of urine collections. This constancy is essential when working at low rates of urine flow, since otherwise the dead space in the ureters and kidney introduces a large error. The periods of urine collection varied from 30 to 40 minutes, and the blood was taken at the midpoint.

The technique of urine collection varied from that employed in previous work reported from this laboratory. The bladder was emptied by means of a silk catheter as completely as possible; then exactly 20 cc. of water were introduced into the bladder and returned into a second graduate. The urine volume was calculated from the first emptying plus any excess obtained in the 20 cc. washout specimen. The washout was then added to the original specimen and the total volume made up to 100 cc. A second dilution was then made to the expected U/P ratio of the inulin. The facts that the clearances are fairly constant, and that the apparent urine flows are likewise constant or vary systematically up or down within a narrow range, are evidence that this method insures complete urine collection with accurate information on the rate of urine flow. Since the plasma levels were low we did not encounter any precipitation of inulin in the urine, despite the high U/P ratio. In the one experiment (see table 1) where this was possible, exactly 20 cc. of water were injected into the bladder at the beginning of each period, to effect immediate dilution of the urine as it came from the ureters. The remainder of the collection procedure was the same as above.

RESULTS AND DISCUSSION. The results of a typical experiment are given in table 1, and the results of all our observations are presented in summary form in table 2. In this series of observations the urine flows have varied from 0.133 to 0.387 and the U/P ratio from 198 to 574. It should be noted that if the U/P ratios are calculated on the basis of concentration per 100

TABLE 1

Comparison of creatinine and inulin clearances in normal dog

Dog B: Basal diet for 42 days (cracker meal, sucrose, butter). The blanks applied to the observed values are: inulin plasma 2.0 mgm. per cent, urine none; creatinine plasma 0.83 mgm. per cent, urine 0.346 mgm. per minute.

PERIOD	URINE FLOW	PLASMA		U/P RATIO		CLEARANCE		CLEARANCE RATIO
		Inulin	Creatinine	Inulin	Creatinine	Inulin	Creatinine	
	cc. per min.	*mgm. per cent*	*mgm. per cent*			*cc. per min.*	*cc. per min.*	
1	0.133	68	7.42	550	546	73.2	72.6	0.99
2	0.133	69	7.60	518	529	69.0	70.4	1.02
3	0.141	67	7.80	516	525	72.8	74.0	1.02
4	0.141	65	7.91	512	522	72.2	73.6	1.02
Average								1.01

TABLE 2

Summary of comparisons of creatinine and inulin clearances

These figures have been corrected for endogenous blanks (see chemical methods).

DOG NUMBER	NUMBER OF PERIODS	AVERAGE URINE FLOW	AVERAGE PLASMA LEVEL		U/P RATIO OF INULIN		U/P RATIO OF CREATININE		AVERAGE CLEARANCE		RATIO OF CREATININE TO INULIN CLEARANCE	
			Inulin	Creatinine	Variation	Average	Variation	Average	Inulin	Creatinine	Variation	Average
		cc. per min.	*mgm. per cent*	*mgm. per cent*					*cc. per min.*	*cc. per min.*		
B	4	0.137	67.6	7.7	550–512	524	546–522	530	71.8	72.6	1.02–0.99	1.01
B	4	0.216	51.1	9.9	393–313	353	381–310	345	76.2	74.5	0.99–0.96	0.98
30	4	0.251	26.5	14.1	410–287	349	430–322	363	87.6	91.1	1.09–0.98	1.04
30	4	0.186	36.4	6.09	440–574	504	452–562	516	93.7	96.0	1.09–0.97	1.02
C	4	0.397	66.6	19.1	210–235	218	209–225	214	86.5	85.0	0.99–0.97	0.98
D	4	0.154	27.9	7.94	562–474	512	518–441	483	78.8	74.4	0.91–0.96	0.95
D	4	0.255	53.3	8.55	496–263	351	462–273	344	89.5	87.7	1.06–0.93	0.98
Mean $\frac{\text{creatinine}}{\text{inulin}}$ clearance ratio												0.99

Corrections for endogenous values applied in calculating above table:

Dog B. See heading on table 1.

Dog 30 (mixed diet)—Inulin-plasma 3.0 mgm. per cent, creatinine plasma 1.09 mgm. per cent, urine 0.487 mgm. per minute.

Dog C (basal diet). Inulin-plasma 2.0 mgm. per cent, creatinine plasma 0.95 mgm. per cent, urine 0.33 mgm. per minute.

Dog D (mixed diet). Inulin-plasma 3.0 mgm. per cent, creatinine plasma 1.0 mgm. per cent, urine 0.508 mgm. per minute.

cc. of water, instead of per 100 cc. of solution, all the ratios reported here will be significantly raised.

The extraordinary magnitude of the U/P ratio of inulin at low urine flows in the dog is noteworthy. The most significant point, however, is the identity of the inulin and creatinine clearances at all rates of urine formation. We have found this identity obtains at rates of urine formation above 1 cc. per minute (Shannon, 1935), and our present data indicate a similar identity down to the lowest rate of urine formation obtainable in a normal dog under the experimental conditions. We believe that this identity is satisfactory evidence on which to preclude the back diffusion of either creatinine or inulin in the normal kidney, since it is highly improbable that the normal tubule cells would permit a molecule as large as inulin to diffuse at the same rate as a small molecule such as creatinine. If any diffusion occurred, the creatinine clearance would drop below the inulin clearance to an appreciable extent as the rate of urine formation fell to low values. This does not preclude the possibility of back filtration, as might conceivably take place thru the capsule in the formation of lymph, where diffusion would not be the determining factor. The mean of the creatinine/inulin clearance ratios in this study is 0.993, with maximum variations of +0.097 and −0.083 and a standard deviation of 0.048 as compared with a mean of 0.994 with maximum variations of +0.086 and −0.064 and a standard deviation of 0.034 obtained in the previous study.

The relationship between the rate of urine excretion and the inulin (or creatinine) and urea clearances will be discussed in a subsequent paper.

SUMMARY

A comparison of inulin and creatinine clearances in dog has been made at low rates of urine formation, the U/P ratio of inulin varying from 198 to 574. In a series of 28 observations on 4 dogs the ratio of the simultaneous inulin and creatinine clearances has a mean of 0.993 with a standard deviation of 0.048 and maximum variations of +0.097 and −0.083.

This identity of clearance in the presence of very high concentration ratios is interpreted as indicating there is no passive reabsorption (diffusion) of either substance during their excretion by the normal dog kidney.

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THE PHYSIOLOGICAL MECHANISMS INVOLVED IN THE CLINICAL MEASURE OF PHASE ANGLE

Circuits Simulating the Phase Angle Properties of the Body; Correlations with Experimental Findings in Normal and Pathological States

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Historical. Since the work of Vigouroux (1) in France, of Lueg (2) and his co-workers in Germany and of Brazier (3) and Northfield (4) in England, it is well established that the body undergoes marked changes in its electrical properties when thyroid function is disturbed.

Credit is due to Lueg for the first clinical applications of importance in this field. Utilizing a technique whose broad lines were laid down by Gildemeister (5), he was able to show first that the electro-cardiographic changes in myxedema are in part due to modifications in the electrical properties of the skin and then, in collaboration with Grassheim (6), that these electrical changes can be correlated with the B. M. R. both in hypothyroid and in hyperthyroid states. However, it was Brazier who, in 1933, perfected a simple and rapid clinical method for measuring thyroid function in terms of phase angle, a factor expressing the relation between the resistance and the capacity of the body. Her work has since been confirmed by Holiday and Smith (7), and, in part, by Robertson and Wilson (8).

Definition of phase angle. Assume an alternating current traversing a pure resistance such as a nichrome wire (fig. 1a) and an ammeter *A* and a volt-meter *V* connected to the wire to show the effect of the latter on the current and voltage. If the alternating currents traversing these instruments be made visible in an oscillograph they will be found to be superposed (fig. 1b), i.e., the current and voltage will appear in step or phase and the phase angle between them 0°. Now, suppose the nichrome wire removed and an air condenser or a Leyden jar put in its place (fig. 1c). The picture of the current and voltage will then take the form shown in figure 1d, i.e., they will be out of step or phase and, if the condenser be a good one, the angle between the current and the voltage will be found to be 90°. The human body acts neither like the pure resistance in figure 1a nor like the condenser in figure 1c. When alter-

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

nating current passes therethrough, the current and the voltage take an intermediate phase relation (fig. 1f) which for normal females is about 6° and for normal males about 8°. This means that the body contains both resistance and capacity (figs. 1e and 1g) arranged in series or parallel or both and that the resistance effect is much greater than that of the capacity (small phase angle).

If the resistance and capacity be assumed to be in parallel, the phase angle Φ is expressed by

$$\tan \Phi = R\omega C \qquad (1)$$

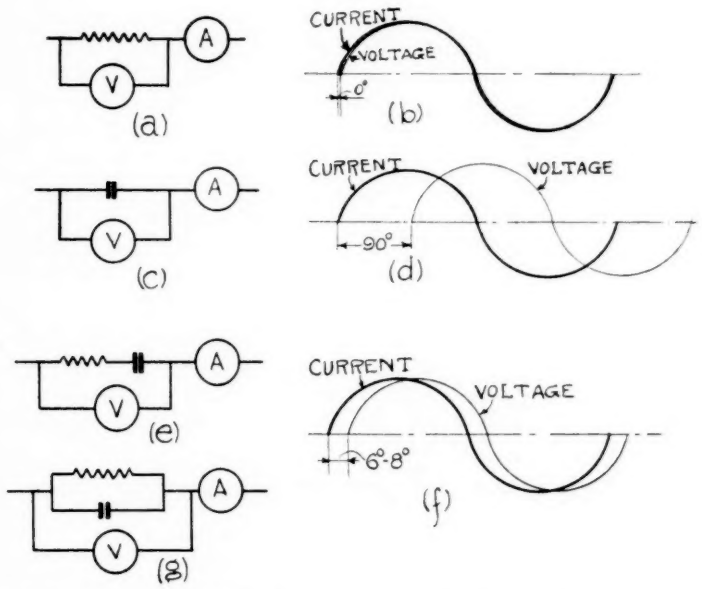

Fig. 1. Phase relations between current and voltage in circuits containing pure resistance, pure capacity and combinations of resistance and capacity.

a, circuit containing pure resistance; *b*, phase relation between current and voltage for circuit *a*; *c*, circuit containing only capacity; *d*, phase relation for circuit *b*; *e*, circuit containing resistance and capacity in series; *f*, phase relation for circuits *e* and *g*; *g*, circuit containing resistance and capacity in parallel. Note: For purposes of illustration the current and voltage in *b* are shown slightly out of phase. These curves should be superposed.

where R and C are resistance and capacity respectively and ω is $2\pi x$ frequency of the alternating current used. In the case of a series arrangement of resistance and capacity

$$\tan \Phi = \frac{1}{R\omega C} \qquad (2)$$

Technique of phase angle measurement. The subject to be measured immerses both arms to a point above the elbows in 10 liters of normal saline contained in a pair of arm-baths, and pure sinusoidal alternating current 9,000 or more cycles in frequency

is led via a pair of lead electrodes into the saline baths. The resistance and the capacity of the subject are then measured by means of a Wheatstone bridge and the values, substituted in equation (1), give the tangent of the phase angle. Since the tangent of a 6° to 8° angle and the angle itself differ but little, Brazier has taken the value of the tangent as representing that of the angle.[2]

The phase angle may also be measured by means of a phase-meter (to be described elsewhere) utilizing a wattmeter or a cathode-ray oscilloscope to indicate the relation between current and voltage.

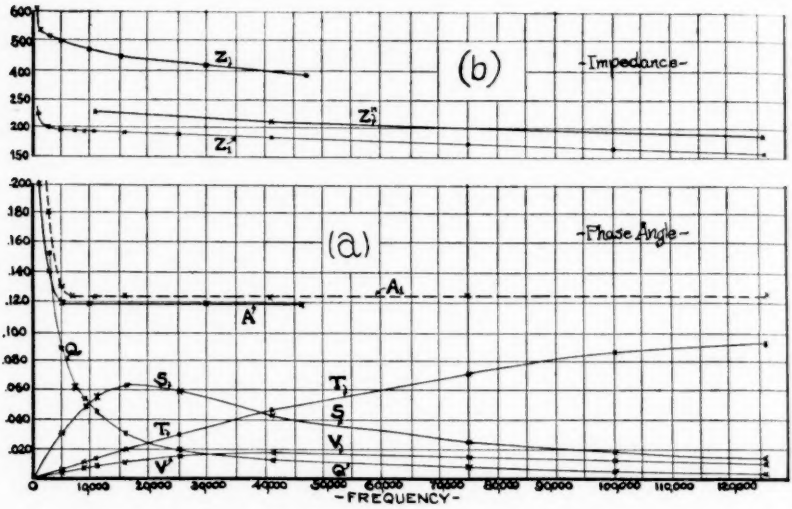

Fig. 2. Phase angle and impedance as a function of frequency.

a, abscissae in cycles, ordinates in tangents of phase angle; *b*, abscissae in cycles, ordinates in ohms (note that the scale in ohms for curve Z is different than for curves Z_1 and Z'').

Phase angle as a function of frequency. Were the body a simple combination of resistance and capacity arranged in parallel or series as in figures 1e and 1g, the phase angle would increase or decrease indefinitely with increasing frequency (see equations (1) and (2) above). Actual measurements by Brazier show that, in normal individuals, the phase angle first decreases with frequency (curve A, fig. 2) up to 5,000 cycles and then becomes

[2] The tangent of the phase angle is, what is termed, the Q-factor in current accepted electrical terminology. Brazier has chosen to express her results in terms of the complement of the phase angle which, in England, is called the Impedance Angle. This latter term is not in general use in this country. She appears to have been led to adopt the term Impedance Angle because the body was supposed by her to act like a pure dielectric under the conditions of measurement. However, as will appear further on in this article, there are grave reasons for doubting that the body acts like a pure dielectric.

constant, the range of constancy extending to 48,000 cycles, the upper limit of frequencies employed.[3] The descending portion of the curve indicates that the body contains somewhere the equivalent of a series arrangement of resistance and capacity, the constant portion, that some parallel arrangement is also involved acting to counteract the series effect.

It is evident that, if a circuit could be found whose phase angle varied with frequency in the same manner as that of the body, some insight might be obtained into the mechanisms involved in phase angle determinations and a more substantial groundwork might be provided for the further study of the electrical properties of the body.

It is the purpose of the present investigation to present three such circuits and to correlate their properties so far as possible with experimental and clinical findings.

The three simulating circuits. These are shown in figure 3. The circuits in figures 3a and 3b designated as of the A- and B-types respectively are in all respects equivalent, each of the resistance and capacity elements beyond *C*, *R* in the B-type of circuit being calculated to have the properties of the corresponding resistances and capacities in the A- type. An analysis of the A-type of circuit will, therefore, suffice for both.

Consider this circuit divided into sections *q*, *s*, *t* and *v* (fig. 3a). It can be shown that the phase angle Φq, Φs, Φt, and Φv for each section at any frequency (neglecting shunting resistance *R*) is given by

$$\Phi_q = XG \tag{3}$$

$$\Phi_s = \frac{X_1}{(R_1^2 + X_1^2)G} \tag{4}$$

$$\Phi_t = \frac{X_2}{(R_2^2 + X_2^2)G} \tag{5}$$

$$\Phi_v = \frac{X_3}{(R_3^2 + X_3^2)G} \tag{6}$$

where G is the reciprocal of the resistance for the complete circuit and X, X_1 X_2 and X_3 the reactances of capacities **C**, C_1, C_2 and C_3 $\left(\frac{1}{C\omega}, \frac{1}{C_1\omega}, \frac{1}{C_2\omega}\right.$ and $\left.\frac{1}{C_3\omega}\right)$ respectively, the value of G being expressed by

$$G = \frac{1}{R_0} + \frac{R_1}{R_1^2 + X_1^2} + \frac{R_2}{R_2^2 + X_2^2} + \frac{R_3}{R_3^2 + X_3^2} \tag{7}$$

If these equations be developed, Φq will be found to represent a hyperbola (see curve *Q*, fig. 2) and Φs, Φt and Φv curves in which the phase angle increases first from

[3] It will be shown that, because of certain corrections omitted by Brazier, the phase angle frequency curve is not, strictly speaking, as here described.

zero to a maximum with frequency, then decreases to an asymptotic value (see curves S, T[4] and V, fig. 2).

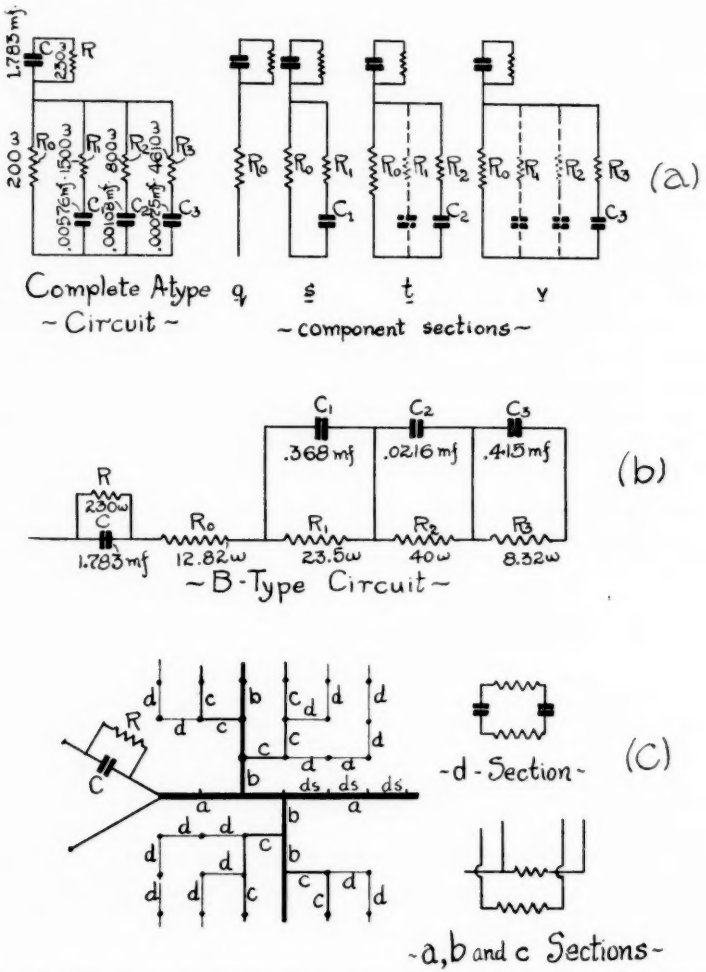

Fig. 3. Circuits simulating the phase angle and impedance properties of the body. *a*, A—type circuit complete (left) and with component sections (right); *b*, equivalent B—type circuit; *c*, infinitely branching type of circuit.

If the various resistances and condensers be given the values indicated in figure 3, and, if the ordinates of the phase angle curves for each section be added at corre-

[4] Only the ascending branch of curve T is shown, the descending branch lying at higher frequencies than those included in this figure.

sponding frequencies (sum of equations 3, 4, 5 and 6) curve A_1, corresponding to the one found experimentally by Brazier is obtained.

The curve showing the variation of resistance[5] with frequency may be plotted from equation (7) by inserting the proper reactance values at each frequency. It has the general form (Z_1, fig. 3b) of curve Z found by Brazier. The impedance curve of Brazier extends only to 48,000 cycles and appears to have been taken on an individual having a small arm section and an exceptionally high body resistance (400–600 ohms). Z^* is a composite curve of the body resistances found in a group of 10 normal individuals having values nearer to that of the simulating circuit. The average arm circumference taken 2″ above the bend of the elbow for this group was 12 inches. Calculated curve Z_1 would correspond to that of an individual having a somewhat larger arm circumference.

In calculating phase angle-frequency curves of the type of A_1 in figure 2, the constant phase angle level for varying frequency may be raised or lowered by increasing or decreasing proportionally resistances R_1, R_2 and R_3 and the point at which the phase angle curve becomes constant with frequency may be shifted laterally by a proportional increase or decrease in the values of condensers C_1, C_2 and C_3.

Impedance curve Z_1 may be given any desired slope by increasing or decreasing resistance R_0 relatively to R_1, R_2 and R_3. The effect of resistance R shunting condenser C may be calculated by means of the usual formulae and, in the particular case of a circuit having the constants in figure 2a, has a negligible effect above 10,000 cycles. Condenser C, as will appear further on, is believed to represent the skin capacity, while resistance R represents its resistance.

The A-type circuit in figure 2a was constructed to test the accuracy of the values calculated. The results are given in table 1 and include nine frequencies for which test apparatus was available.

Phase angles below 10,000 cycles were measured with a Wheatstone bridge using a beat frequency oscillator containing 3 to 4 per cent harmonics as a current source. At the higher frequencies, the special cathode ray type phase meter, already referred to, furnishing substantially harmonic-free oscillations was used. The accuracy of the phase angle determinations at frequencies below 10,000 cycles may be taken as ± 5 per cent. Above this frequency the values are reliable to ± 0.5 per cent up to 41,800 cycles and to ± 2.0 per cent at 126,000 cycles. The measurements at 15,300, 41,800 and 126,000 cycles were selected as corresponding roughly to the peaks of curves S, V and T respectively.

The average calculated phase angle in the range of constancy with frequency (7,500–126,000 cycles) was 0.1233 and the maximum deviation from this value + 1.2 per cent at 15,300 cycles and −1.1 per cent at 41,800 cycles. The values found by test all fall within 1 per cent of those calculated at the frequencies for which harmonic free oscillations were available.

Although the infinite network shown in figure 3c appears to be very

[5] The A.C. resistance of the body differs but little from its impedance and, in a rough way, varies inversely with the cross-section (or the circumference) of the arms. The terms resistance and impedance will be used interchangeably throughout the rest of the article.

different from the A and B-type circuits described, it may, in reality, be considered as an equivalent. It is well known that lump combinations of resistance and capacity such as R_1, C_1, R_2, C_2 or R_3, C_3 in figures 3a and 3b can be approximately imitated by combinations of resistance and capacity of small value spread along infinite transmission lines. These transmission lines yield phase angle-frequency curves somewhat similar to component curve S in figure 2a. By combining four such lines, a simulating circuit is obtained whose properties can be shown to be substantially the same as "lumped" type circuits A and B.[6]

This network, because of its complex nature, could not be constructed for test, but it is of interest as a possible representation of nerves, blood vessels and like structures composed of a main trunk ramifying into branches and all combining to form an impedance having properties similar to the A and B circuits shown in figures 3a and 3b. These latter circuits can also be rendered infinite by adding more sections (parallel or series) and changing the constants accordingly.

While the three simulating circuits shown are represented throughout as the pure A, B or infinitely branching types, it is obvious that equivalent

TABLE 1

Calculated and found phase angle values for test model of simulating circuit in figure 3a

FREQUENCIES IN CYCLES	1000	3000	5000	7500	9000	11,160	15,300	25,000	41,800	75,000	100,000	126,000
Φ calculated	0.390	0.173	0.1310	0.1228	0.1229	0.1227	0.1247	0.1239	0.1219	0.1226	0.1235	0.1244
Φ found	0.383	0.175	0.1320	0.1260	0.1213	0.1230	0.1250		0.1220			0.1253

portions of each could be combined to form mosaics having the properties of the pure forms. For example, since the B-connected elements in the circuits shown in figure 2b are exact electrical equivalents in their impedance properties to the corresponding A-connected elements in figure 3a, heterogeneous simulating circuits composed of successive elements taken from each would have the same properties as those composed of the homogeneous elements shown. Similarly equivalent portions of the infinite network could be combined with A and B-connected elements to simulate the purer forms.

Experimentally found forms of phase angle-frequency curves. Turning to a consideration of curves Q, S, T and V in figure 2 representing component sections of the complete simulating circuits in figure 3a, it will at once be evident that, if the values of capacity and resistance be changed in any one section, the form of the corresponding curve will be altered as to slope or curvature at various points so that when the total effect of all the

[6] The mathematical derivation of the properties of this network is given in an appendix.

curves is added, curve A_1, representing their sum, will no longer remain constant with frequency in the required range but will be deformed, the deformation taking place along those portions most affected by the change. Similarly, if the resistance and capacity of all of the sections be changed, curve A_1 can be made to take an ascending, a descending or an irregular form. In the expectation that individuals could be found in whom these conditions had been realized physiologically, phase angle determinations were made at four spaced frequencies (11,160; 15,300; 41,800 and 126,000 cycles) in a search for various types of curves. Figure 4 shows examples of those found. The descending form, curve *I*, is the rule in normal individuals but appears to be accentuated in subjects suffering from certain pitu-

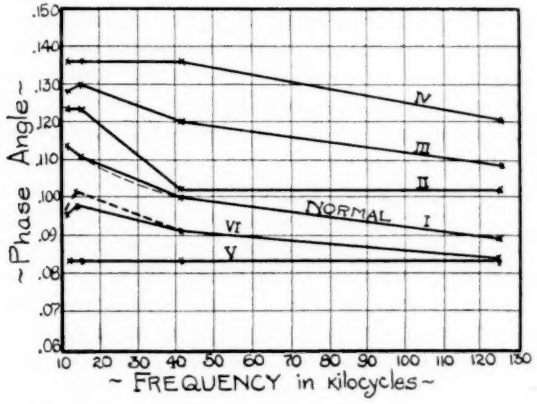

Fig. 4. Forms of phase angle-frequency curves.

I, composite of 30 normal individuals; *II* and *III*, less frequent normal forms; *IV*, achondroplasic individual; *V*, hyperthyroid individual showing constant phase angle with frequencies up to 126,000 cycles. In *I* the dotted line shows the probable course of the curve. In *VI* the dotted line indicates the change occurring at periods.

itary disturbances. Less frequently one finds those of a broken type-*II*, or peaked at 15,300 cycles-*III*. An achondroplasic yielded a curve constant with frequency up to 41,800 cycles-*IV*. The only individual found to have a constant phase angle up to 126,000 cycles was not normal-*V*. This was a case of Graves' disease which, when first measured, gave a phase angle-frequency curve of type *I*, figure 4. After 10 days of iodine medication given by the transcerebral ionization technique of Bourguignon (9) and intended to modify pituitary function, the curve became horizontal and remained so over a period of a week, returning thereafter to type I. Studies of the variations of phase angle with immersion are given for this individual further on.

These various types of curves show that the phase angle measured above

5,000 cycles may vary with frequency under both normal and pathological conditions. As already pointed out, the simulating circuits described may be fitted to any form of curve by changing the constants of its component sections.

While the original phase angle-frequency curve given by Brazier (3) was of the form shown in figure 2a, it does not truly represent the relation under comparison since this author states that no corrections were made for lead-electrode effects.[7] The lead cables and the arm-baths introduce a certain amount of capacity and have the effect of raising the phase angle values as the frequency increases. For example, in the authors' apparatus, at 41,800 cycles this correction varied from 3 to 5 per cent using cables having a total length of 3 feet and with arm-baths spaced one foot between center lines. The true phase angle-frequency curve for the normal human body should, therefore, have had a downward slope in the range represented as constant with frequency. The average phase angle at 11,160, 15,300, 41,800 and 126,000 cycles in 30 unselected individuals having a normal basal metabolism and normal phase angle was found to be 0.114, 0.111, 0.100 and 0.089 respectively (fig. 4, *I*). The total difference in the range 11,160 to 126,000 cycles was approximately 25 per cent after all corrections for leads, arm-baths and body to ground had been made.

In certain individuals, the phase angle-frequency curve is found to vary in form and position over a period of time It is shown in a paper to be published elsewhere that, in certain types of pituitary disturbances, the absolute value of the phase angle taken at a single frequency is subject to considerable variation in the course of a month, normal subjects, according to Brazier, showing no such variations. It has been found that these variations extend along the phase angle frequency curve, the latter sometimes moving upward and downward parallel to itself in the course of a month, but more frequently showing greater variations at one frequency than at others. Curve *VI*, figure 4, shows variations in one case of this kind where, in the course of three successive menstrual periods, a small peak at 15,300 cycles (solid line) occurring in the intermenstrual period,

[7] Brazier (10) has reduced the original normal phase angle values to be applied at 9,000 to 20,000 cycles because of an inadequate correction for body capacity to ground. This correction increases with frequency and, if originally applied to the phase angle-frequency curve, was probably also inadequate.

The effect of the electrode phenomena would be to alter the form of the curve at frequencies below 10,000 cycles since it is at these frequencies that they become marked. It will be noted that the curve calculated from the simulating circuit constants is slightly displaced laterally with respect to Brazier's. By increasing the capacity of C_1, C_2 and C_3, as already stated, the two curves can be brought into superposition. However, it was not considered to be important to obtain exact superposition in view of the uncertainty of the exact form of curve A after correction for electrode phenomena.

accentuated itself during each period, the values at other frequencies remaining substantially stationary throughout. As already noted, in a case of Graves' disease, the phase angle-frequency curve changed from type *I* to type *V*, figure 4, and back to type *I* in the course of treatment. This individual also developed a peak of 15,300 cycles in the course of one menstrual period.

Significance of certain portions of the simulating circuits: The rôle of the skin: Experimental confirmation of the value chosen for condenser C. It will be noted that the three simulating circuits in figure 3 have only condenser *C* and shunt resistance *R* in common and that these elements are in series with the circuits to which they are attached. In order that component curve *Q* have a hyperbolic form of sufficient steepness to give curve A_1 the proper slope at low frequencies, condenser *C* had to be given a capacity (1.783 mfd) considerably greater than any of the others in the circuit. Inasmuch as, under the conditions of measurement, the current passes from the saline solution in which the arms are immersed through the skin and into the body, it seemed reasonable to assume that condenser *C* represented the skin, and an attempt was made to determine the order of magnitude of the skin capacity by immersing increasingly large skin areas while maintaining all other conditions constant. Since direct current cannot pass through a condenser and since the skin permits such passage, it was necessary to shunt resistance *R* around this condenser to indicate the direct current path.

For a series circuit such as condenser *C* and the elements appended thereto, the phase angle Φ is expressed by equation (2) above. This equation may be written in terms of reactance $\frac{1}{C\omega} = R\,\Phi$.

It suffices, therefore, to determine R and Φ to calculate differences in reactance at different immersion levels and, from these differences, the corresponding skin capacities.

In figure 5a and 5b, phase angle and resistance respectively are plotted against depth of immersion in inches measured above and below the bend of the elbow, while in figure 5c, the reactance R Φ is plotted against the resistance measured at successive immersion levels. Subject 1 was a normal female, age 26; 2, a normal male, age 32; 3, an athyroid (totally thyroidectomized) individual, 47 years of age; 4, a hyperthyroid female, age 28, and 5, a male achondroplasic, aged 14 years.

The phase angle-immersion curves for the two normal subjects are of interest as showing a plateau for all frequencies at points lying between 1 inch and 2 inches above the elbow. It is noteworthy also that the phase angle at 11,160 cycles is lower at points just below the elbow than above the latter, whereas the reverse is true at the higher frequencies. The steeply

descending portions of the curves at immersions high on the arm[8] are found only in subjects having moderately thin arms, subjects 1 and 2 having arm circumferences (at 2 inches above the bend of the elbow) of 8 inches and $9\frac{1}{4}$ inches respectively. With larger arms, the immersion curve tends to

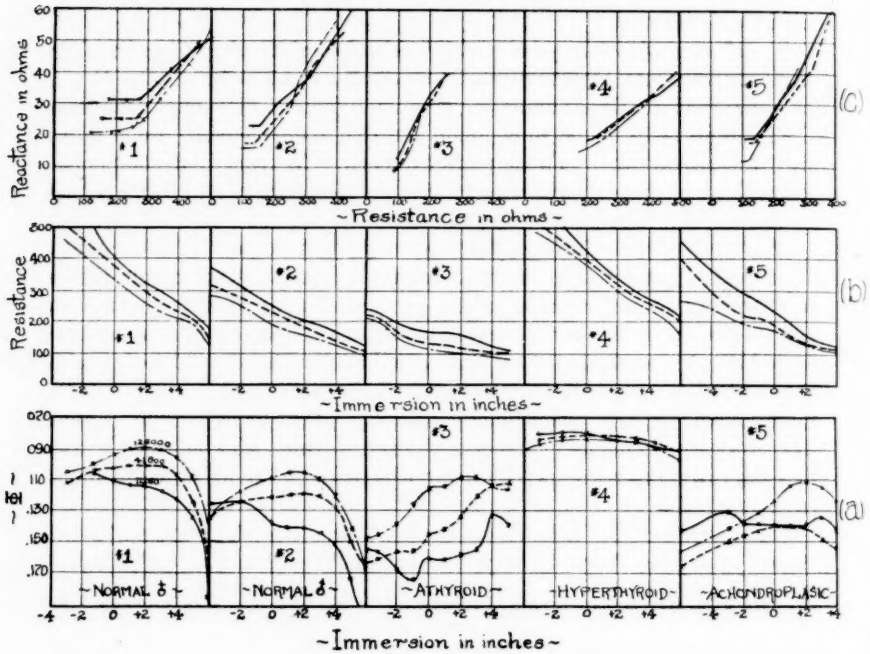

Fig. 5. Effect of varying immersion on phase angle, resistance and reactance.

(a), abscissae, immersion in inches measured relatively to the bend of the elbow as O, distances below the elbow being designated as minus and above the elbow as plus, ordinates in tangents of phase angles; (b), abscissae in inches immersed as in (a), ordinates in ohms, resistance; (c), abscissae in ohms resistance, ordinates in ohms, reactance obtained by multiplying resistance by phase angle at each immersion level. Values obtained at 11,160 cycles are shown throughout as a solid line, while those for 41,800 and 126,000 appear as dotted and dot and dash respectively in the manner indicated for subject 1 in (a). The phase angle, resistance and reactance curves for each subject are arranged vertically in (a), (b) and (c).

take the flattened form shown for subject 4 who had an arm circumference of $10\frac{7}{8}$ inches.

[8] For high immersions the volume of saline in the arm-baths was increased from 10 to as much as 14 liters. This was found to have a negligible effect on the phase angle values. It was also found necessary to use long lateral lead electrodes extending the whole length of the arm baths instead of the short end electrodes recommended by

The curves for athyroid subject 3 follow an upward, instead of a downward, course as immersion is continued beyond the plateau value at +2 inches. The extreme irregularity of these curves contrasts with the smooth immersion curves of the normal subjects. Were subject 3 normal, his immersion curve should have taken the flat regular form of subject 4, since the arm circumference was $14\frac{3}{4}$ inches.

The curves for subject 4 are given not only as an example of what is encountered in hyperthyroidism, but because this individual at the time of the test, presented type *V* (fig. 4) phase angle-frequency curve (constancy with frequency up to 126,000 cycles). The phase angle values all lie within 1 per cent at +2 inches and diverge but little at the extremes of immersion.

The curves of achondroplasic 5 show the values at 11,160 cycles lying between, instead of below, those at the two higher frequencies except for points on both sides of +1 inch where the 11,160 and 41,800 values coincide. The low frequency curve is of interest as presenting two peaks on either side of the +1 inch to +2 inch plateau. The arm circumference of this boy was 10 inches.

The resistance-immersion curves in figure 5b present nothing of note except in the case of subject 4. The slope of the resistance curves generally follows the size (section) of the arm, thin arms showing steeper slopes and higher resistances at corresponding immersion levels. In the case of subject 4, the resistances are uniformly higher than for normal subject 2, though the arm circumference was greater ($10\frac{1}{2}$ inches against $9\frac{1}{4}$ inches). It may be said, however, that high arm resistance of this kind is not the general rule in thyrotoxicosis.

The resistance-reactance curves in figure 5c present three noteworthy features: 1, those of subjects 1, 2 and 5 become asymptotic to the resistance axis at resistances corresponding to high immersion levels; 2, the curves for athyroid subject 3 and of hyperthyroid subject 4 appear to represent opposite extremes of slope as compared to normals 1 and 2; and 3, the sloping portions of all the curves show segments directed towards the origin.

The existence of asymptotic portions on these curves (subjects 1, 2 and 5) may be taken to mean that the total capacity of the skin increases with the area immersed until the capacity values are of sufficient magnitude to render the skin reactance $\left(\frac{1}{C\omega}\right)$ negligibly small, the residual reactance, read off where the horizontal portion of each curve cuts the axis of ordinates,

Brazier. This was done to shorten the path between arm and electrode and eliminate the effect of electrolyte resistance amounting to 25–30 ohms. Tests made with these long electrodes using the simulating circuit model as an artificial body showed the phase angles to be closer to the true values than with the short standard electrodes particularly at lower frequencies and for low body impedances. The question of electrode size and position will be taken up in greater detail in a subsequent paper.

representing largely that of the thorax or, strictly speaking, those portions thereof in series electrically with the arms. The point at which the skin reactance becomes negligible was found to lie (in subjects 1, 2 and 5) about three-quarters of the way up the arm between elbow and axilla. Below this level, the skin reactance values change rapidly in accordance with the degree of immersion. It will be noted that the resistance-reactance curves of subjects 3 and 4 fail to show an asymptotic portion even at maximum immersion. This is believed to have been due to the fact that the arm circumferences ($10\frac{7}{8}$ inches and $14\frac{3}{4}$ inches respectively) were considerably greater than those of the other subjects and the resistance gradient approaching the axillae not sufficient to bring this out.

The extremes of slope of the curves for athyroid subject 3 and hyperthyroid subject 4 as compared with those of the normals would seem to indicate that the electrical properties of the skin (reactance and capacity per unit change of resistance) may be profoundly modified with changes in thyroid function. Further investigation will show whether or not the slope characteristic of the resistance-reactance curve may be taken as a measure of thyroid function.

The fact that segments of the resistance-reactance curves point towards the origin means merely that, along the segments in question, the phase angle of the skin remains constant.

The original purpose of the studies on reactance changes with varying immersion was to experimentally determine the order of magnitude of the skin capacity for comparison with the value assumed for condenser C in the simulating circuit. The capacity value for this condenser, it will be remembered, was chosen to fit a phase angle-frequency curve of the type originally reported by Brazier (curve *A*, fig. 2) and this curve was plotted from values determined with the arms immersed to a point where the phase angle becomes constant which, as has been shown, lies about 2 inches above the bend of the elbow in normal individuals. To calculate the order of magnitude of the skin capacity at this point, it is merely necessary to subtract the reactance value at maximum immersion (where the skin reactance becomes negligible) from the reactance at + 2 inches and equate to $\frac{1}{C\omega}$. In the case of subject 2, whose body impedance approaches nearest that of the simulating circuits in figures 3a and 3b, the differences in reactance at these two points for frequencies of 11,160, 41,800, and 126,000 cycles were found to be 3.8 ohms, 2.0 ohms and 0.6 ohm respectively. Equating these reactance differences to $\frac{1}{C\omega}$, the corresponding capacities calculated are 3.7, 1.9 and 2.1 mfd. These skin capacity values are of the order of those assumed in the simulating circuit, i.e., 1.783 mfd and support the assumption that condenser C represents the capacity of the skin.

Similar calculations for subjects 1 and 5 show large skin capacity values in spite of the fact that the body impedances differ considerably from those given in the simulating circuits.

Discussion. The simulating circuits herein described are not to be taken as representing the phase angle properties of the body as a whole since their development is based upon data taken from arm to arm measurements. Their interest lies largely in suggesting a picture applicable to the arm-to-arm segment and which may be extended to other parts of the body at some later time by a similar mode of analysis.

The effect of substances having an elective action on the glandular, autonomic, muscular and vascular systems is being studied at various frequencies with a view to determining possible factors controlling the various component parts of the circuits presented and to permit a choice from among the latter of the one best representing the underlying mechanisms involved.

The finding of a phase angle-frequency curve with a downward slope in the range formerly believed to remain constant does not alter the general form of the simulating circuits described but necessitates a different choice of condenser and resistance values in certain portions thereof.[9]

The significance of the various portions of the simulating circuits with the exception of condenser C is, as yet, unknown. In two cases, an increase in the phase angle values was observed at 15,300 cycles during the menstrual periods. From an examination of figure 2a, it will be seen that curve S shows a peak at about this frequency. This suggests that the circuit corresponding to this curve (section s, fig. 3a) is responsive in some way to the mechanisms involved in menstruation (pituitary or ovaries). However, the number of cases in which this phenomenon was observed is too small to permit any conclusions.

Brazier (3) has published immersion curves in which phase angle was plotted against the volume of arm immersed. It is difficult to compare these curves with the ones here reported in which linear distances are used instead of volume. It would appear that the immersions employed by

[9] Thus, by increasing the value of R_2 (fig. 3a, section t) and diminishing the capacity of condenser C_2 proportionally, the slope of component curve T may be reduced so as to give curve A_1, representing the whole circuit, a downward slope fitting the experimental values found. The same result also may be accomplished by changing the values of R_1 and C_1 to increase the slope of component curve S on the downside, or by a change of constants modifying the slopes of both the S and T curves. It has not been considered that data on normal individuals are as yet sufficient (30 subjects) to permit statistical treatment and a choice of definite average values fixing the final form of the curve. When a fair average curve will have been obtained from measurements of several hundred normal subjects made at a larger number of frequencies, and at an immersion level shown to have special significance, the constants of the simulating circuits may be chosen to fit the experimental values so determined.

this author were not pushed to sufficiently high levels to show the inflected portions of the curves.

This worker has minimized the rôle of the skin at frequencies above 10,000 cycles. It is difficult to interpret the resistance-reactance curves in figure 5c otherwise than as showing that the skin is a very important factor at these frequencies since they were obtained by maintaining all conditions constant and varying only the surface of the skin immersed. The finding of an asymptotic (horizontal) portion on these curves may be taken to mean 1, that the skin reactance decreases with immersion until it reaches a limiting value, or 2, that this reactance continues to decrease until it becomes negligible. Preliminary mathematical analysis, assuming the skin to act like a series of infinitely small imperfect condensers strung along a resistance and adding small increments of capacity as immersion is increased indicates that the first of these interpretations is probably the correct one. This question will be taken up in a paper dealing more in detail with the rôle of the skin.

The work on immersion shows that the best level for the routine clinical determination of phase angle is 2 inches above the bend of the elbow. Higher immersions may yield higher values in normal individuals (see curves for subjects 1 and 2 in fig. 5a) particularly if the arms are thin or short, and low values, in thyroid deficient subjects (see curves for subject 3 in the same figure). In certain long-armed individuals the "plateau" phase angle values may be found to lie at about the 3 inch level and should be sought by increasing the immersion.

In the routine measure of phase angle to determine thyroid function, if the immersion level could always be fixed at the point where the sloping and horizontal portions of the resistance-reactance curves intersect, it might then be possible to decide whether phase angle changes in thyroid disease were due predominantly to changes in the electrical properties of the skin (the skin reactance at this point becoming constant) or of other tissues. It has been shown, however, that, in obese or even moderately fleshy individuals, this point is not attained even with immersions up to the axillae. Phase angle measurements as at present made with immersions 2 inches to 3 inches above the bend of the elbow represent the relation between the resistance and reactance at one point on the resistance-reactance curve and tell us nothing as to the slope of the latter nor where (or if) it becomes asymptotic. The values so obtained would seem to include at least two factors of which the skin is one.

The question arises then as to whether it is the effect of the skin or of other structures which predominates in measurements of this kind. Examination of component curve Q (fig. 2a) representing the effect of the skin at each frequency would seem to indicate that, in the vicinity of 10,000 cycles, the skin accounts for a considerable part of the total phase angle and that

at lower frequencies its proportional effect is even greater. It is significant that Lueg, the pioneer in this field should have operated at low frequencies (1400 cycles) and that he should have found, with less perfect technical equipment, changes in the electrical properties of the skin accompanying thyroid disturbance.

APPENDIX. *Mathematical derivation of the properties of the network in figure 3c.* In a simple transmission line *a* having a resistance per unit length R and a susceptance per unit length $j\omega C$, the surge impedance

$$Z_s = \sqrt{\frac{z}{y}}$$

where z is the impedance per unit length and y the corresponding susceptance: From which it follows that

$$Z_s = \sqrt{\frac{R}{j\omega C}} = \sqrt{\frac{R}{\omega C}} \, (\text{Cos} - 45^\circ + \text{j sin} - 45^\circ)$$

If now a second transmission line *b* be joined to line *a* and the admittance of line *b* per unit length be made the same as the surge impedance Z_s of line *a*, the surge impedance of line *b*, Z_t, can be shown to be

$$Z_t = \sqrt{R \sqrt{\frac{R}{\omega C}}} \, (\text{Cos} - 22.5^\circ + \text{j sin} - 22.5^\circ)$$

Assuming this same process to be continued two stages further so that each increment of admittance over unit distance *ds* for a third line *c* is equal to Z_t and the corresponding admittance increment of a fourth line *d* equals the surge impedance of the third line, a mosaic is obtained such as is shown in figure 3c whose total surge impedance Z_m is expressed by

$$Z_m = \left[\frac{R^{15}}{\omega C}\right]^{\frac{1}{16}} (\text{Cos} - 5\ 5/8^\circ + \text{j sin} - 5\ 5/8^\circ)$$

which is the order of magnitude of the phase angle of the body.

To obtain the necessary admittance values, the impedance per unit length for lines *a*, *b*, *c* and *d* should have the properties of the units indicated in figure 3c.

It has been shown that the phase angle decreases somewhat with frequency. This network can be given this characteristic by using a composite of only three infinite lines instead of four and by introducing the necessary leakage.

SUMMARY AND CONCLUSIONS

1. Three circuits are presented capable of simulating the phase angle properties of the body in normal and pathological states.

2. The normal values for phase angle have been found to diminish with increasing frequency in the range formerly believed to remain constant.

3. Phase angle measurements have been extended to 126,000 cycles

and the values at this frequency found to be about 25 per cent lower than at 11,000 cycles.

4. Phase angles obtained by the immersion method are shown to reach a plateau value one to three inches above the bend of the elbow and to increase, in normal individuals, with immersion above this level.

5. In an athyroid individual, the phase angle decreased at high immersion levels instead of increasing.

6. Phase angle determinations by the immersion method are shown to include at least two factors of which the skin is one.

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METABOLIC STUDIES IN OSTEOPOROSIS

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Although there have been many investigations on the value of calcium salts in correcting a deficiency of calcium, there have been few accurate quantitative comparisons of the influence of these salts on the retention of calcium. It has been shown frequently that when large amounts of relatively soluble calcium salts are administered, sufficient calcium is absorbed to produce definite although temporary changes in the concentration of calcium in the blood. In conditions in which abnormally low values for calcium in the blood are found, it also has been possible to produce a gradual elevation in the basal level of calcium by means of administering these salts. Only a few investigations have been reported in which the relatively insoluble calcium salts were used, and the changes in the concentration of calcium in the blood produced by them were small and variable. In general, the efficiency of the different calcium salts in producing these changes in the blood seems to depend on their relative solubilities, but it is impossible from these studies of the blood alone to obtain any quantitative information as to their actual utilization.

In studies on the calcium balance of human subjects, calcium chloride and calcium lactate (9, 15, 23, 28, 29, 33, 38) have been most frequently used and they appear to be valuable sources of calcium, especially when given in large amounts. Calcium acetate, calcium glycerophosphate (15) and calcium gluconate (2) have also been shown to produce an increased retention of calcium. In general, a parallel increase in the retention of phosphorus has been observed. A positive balance from administration of tribasic calcium phosphate alone has not been demonstrated, but the administration of tribasic calcium phosphate with cod liver oil has been found to produce a marked improvement in the retention of calcium and phosphorus in cases of rickets (14) and in a case of osteomalacia (25). Blühdorn and Grosser are the only investigators who have reported the results of feeding more than one of these calcium salts to the same individual under similar dietary conditions. Blühdorn has reported balance studies on a child fed calcium chloride and calcium lactate, 56.6 per cent of the calcium being retained when calcium chloride was given and 45.0 per cent being retained when calcium lactate was given. The amounts of

these salts fed were approximately equivalent. Although Grosser has reported the results of giving more than one calcium salt to the same individual, his studies provide little information as to the relative efficiency of the various calcium salts in producing a positive calcium balance because of variations in the method of administering the salts and in the amounts given.

The results of balance studies on animals (24, 35, 36), as well as of the changes produced in the rate of growth (34) and the ash of these animals (26), in general seem to indicate that the calcium of the chloride, lactate, gluconate, carbonate, silicate, sulphate, and dibasic and tribasic phosphates can be utilized if these salts are provided in adequate amounts. It appears necessary to provide rather large amounts of calcium in any form to produce an appreciable storage of calcium, and the results obtained with relatively insoluble calcium salts have been less consistent. It is also impossible from these investigations to obtain any comparison of the relative utilization of the different salts.

The influence of vitamin D on the metabolism of calcium and phosphorus has been the subject of a vast number of investigations, and the recent reviews of Harris (17, 18, 19, 20), Bills, and Schmidt and Greenberg summarize the present information on this subject. There seems to be considerable evidence that under many conditions vitamin D increases the retention of calcium. This is usually produced by a decrease in the fecal calcium, but it has not been possible to determine whether the vitamin produces this effect by increasing the absorption of calcium or by decreasing its re-excretion in the lower part of the intestinal tract. According to Brown and Shohl both the absolute and the relative amounts of calcium and phosphorus in the diet determine the retention of these elements, and vitamin D controls their intermediary metabolism. The beneficial value of vitamin D has been demonstrated chiefly in rickets, and appears to be most important for the growing child or animal. The vitamin D requirement is apparently less when the intake of calcium and phosphorus is adequate, and it appears to be relatively small for adults. In normal adults (5, 12, 21, 22) there is little evidence that vitamin D exerts any influence on the retention of calcium and phosphorus. Its value in producing an increased retention of calcium has been demonstrated for adults with osteomalacia (3, 25), hypoparathyroidism (6), and osteoporosis (3) due to a prolonged dietary lack of calcium.

Our investigation was undertaken to compare the utilization of calcium in calcium lactate and in tribasic calcium phosphate. While the former is a fairly soluble salt and the latter a relatively insoluble salt of calcium, it is obvious that the phosphorus content of the latter might favorably compensate for the fact that it is relatively insoluble, especially if after absorption the calcium and phosphorus would be rapidly removed from

the circulation by deposition as bone; such rapid formation of bone would only be anticipated in a condition like osteoporosis, which had arisen as a result of a long continued negative balance of calcium and phosphorus. The added effect of viosterol on the retention of these salts was also investigated. The subject studied was a woman sixty-five years old who had a senescent type of osteoporosis of high degree, as shown by roentgenologic examination. Her dietary history seemed to indicate that for years the calorific value of her food had been low. The intake of calcium and phosphorus had also been low, although her intake of vitamins and proteins may have been adequate. Low figures were also found for free and total acidity of the gastric juice after stimulation with histamine.

During the nineteen weeks of the experimental study the subject was kept on an intake of fluid of approximately 2000 cc. daily and on a constant weekly diet prepared with great care by a skilled dietitian in a diet kitchen exclusively reserved for this purpose. Three different diets (diets I, II, and III, table 1) were given during each week, an exact duplicate of each of these diets being dried in a current of warm air at about 70°C. and analyzed for nitrogen, sulphur, phosphorus, calcium, magnesium, potassium, and sodium at intervals throughout the study to provide exact information as to the composition and the extent of the variation in composition occurring in the foods used. The patient was most coöperative and only one correction was necessary for uneaten food throughout the entire investigation. This was obtained by direct analysis. This patient's general clinical condition definitely improved on the strict experimental regimen. The total excretion of urine for each twenty-four hours was collected and aliquot parts were analyzed daily for preformed creatinine and for total nitrogen. Acidified aliquot parts were mixed to obtain the specimens for the determination of the weekly excretion of calcium, magnesium, sodium, potassium, phosphorus, and sulphur. The feces were collected in weekly periods and hydrochloric acid was added to prevent loss of nitrogen. Instead of using a marker for separating the feces, an enema of distilled water was used to close each period. The feces were dried in a current of warm air at about 70°C. and were ground to obtain suitable aliquot parts for the determination of nitrogen, sulphur, phosphorus, calcium, magnesium, potassium, and sodium. A muffle kept below 500°C. was used to ash the samples for the inorganic determinations, and the samples were ashed in platinum dishes. The values for calcium and phosphorus in the salts added to the diet were also determined by direct analysis.

The following analytical methods were used: 1, for total nitrogen, macro-Kjeldahl; 2, for phosphorus, Fiske and Subbarow; 3, for total sulphur in food and feces, Berthelot's method (as described by Sherman, 32) based on oxidation in the bomb calorimeter and gravimetric determination as barium sulphate; for total sulphur in urine, Benedict; 4, for calcium, Tis-

dall and Kramer; 5, for magnesium, precipitation as $MgNH_4PO_4$, according to Tisdall and Kramer, and colorimetric determination of the phosphorus by the method of Fiske and Subbarow; 6, for potassium, precipitation with sodium cobaltinitrite and titration with potassium permanganate, according to Tisdall and Kramer; 7, and for sodium, Butler and Tuthill's application of the method of Barber and Kolthoff.

The investigation extended over nineteen consecutive weeks. For purposes of description it can be divided into seven series: Series I was the control and consisted of three weekly periods during which the daily average of the basic weekly intake of calcium was 0.41 gram and of phosphorus 0.68 gram. These amounts of calcium (31) and phosphorus (30) might be expected to maintain equilibrium in an individual weighing 40 kgm. Because of the variation in the amounts of calcium and phosphorus different individuals need to maintain a satisfactory equilibrium, Sherman (30, 31) has recommended an intake of calcium and phosphorus considerably in excess of these amounts to allow a suitable margin of safety, but for this investigation it was not desirable to have a large excess of either calcium or phosphorus in the basic diet. Series II consisted of three weekly periods during which the patient received daily 3.02 grams of calcium and 1.48 grams of phosphorus, in the form of tribasic calcium phosphate, in addition to the control diet. Series III consisted of two weekly periods, with 30 minims of viosterol daily in addition to the tribasic phosphate. Series IV was a second control of two weeks' duration on the basic diet with nothing supplementary added. Series V consisted of three weekly periods with 3.02 grams of calcium added as calcium lactate. Series VI also consisted of three weekly periods with the addition of 1.51 grams of calcium as calcium lactate. Series VII likewise lasted for three weekly periods with 1.51 gram of calcium as calcium lactate plus 30 minims of viosterol. The calcium phosphate was given suspended in water and the calcium lactate was completely dissolved in water. Both salts were given at approximately one-half hour before meals.

Table 1 gives the actual components of each diet and table 2 summarizes the results of the analysis of food. The maximal variations observed as the result of three separate analyses of each of the three diets are given as well as the average values for each. The figures for sodium do not include the extra salt used, the latter being determined separately from analysis of the salt and from the weight of the salt used in each individual period. The actual intake of salt was not absolutely constant from week to week, but was approximately 1.5 to 2.0 grams of sodium daily.

The completeness of the collection of the twenty-four hour specimens of urine was checked by determining daily the values for preformed creatinine. These values were approximately constant throughout; the average daily values for creatinine nitrogen for the individual periods varied from 0.25 to 0.28 gram, the majority of the values were 0.27 gram.

In addition to calcium and phosphorus balances, we have determined balances for potassium, sodium, magnesium, sulphur, and nitrogen. No attempt was made to allow for the excretion of any of these substances through the skin, and in the case of sodium and nitrogen the amount lost

TABLE 1

Constituents of various diets

	DIETS, GM.		
	I	II	III
Orange	100	100	100
Bread, salt free	80	80	80
Butter, salt free	70	70	70
Cornflakes		5	
Cream, 20 per cent	55	40	55
Sugar	25	25	25
Tomato, raw	50		
Carrots	50		
Apricots, canned without sugar	75		
Grapefruit	75		
Potato	150	150	150
Veal chops	50		
Chicken	50		50
American cream cheese	25	25	25
Beer	350	350	350
Celery		10	
Beets		50	
Parsnips		50	
Pineapple, canned without sugar		75	
Banana		50	
Fish		50	
Egg		One	
Milk		20	
Chinese cabbage			25
Squash			50
Peaches, canned without sugar			75
Pears, canned without sugar			75
Lamb chops			50

in one way or another through the skin may significantly decrease the magnitude of the positive balance.

Throughout the entire experiment, lasting nineteen weeks, the positive balance of calcium and phosphorus was in the ratio of 2.2 to 1, a value approximately that in bone. Outside of bone there are no known storehouses for any considerable amounts of calcium, and it is presumable that all, or nearly all, of the calcium retained was deposited as bone. While the

ratio of 2.2 to 1 supports this idea, the closeness of the figure to that for bone may be somewhat fortuitous, as unfortunately, at the end of the experiment, it was impossible to hold the patient longer for a third control period on a standard diet to permit the gastro-intestinal tract and the body to return to the original state of equilibrium. No attempt was made to correct the calcium to phosphorus ratio for the amount of phosphorus retained in the newly deposited protein, as evidenced by a positive nitrogen balance and gain in weight. In view of the varied functions of phosphorus in the body as well as the difficulty in correcting for the amount of

TABLE 2

Chemical analysis of diet

	DIET I, GM.		DIET II, GM.		DIET III, GM.		DAILY AVERAGE FROM WEEKLY INTAKE	
	Range	Average	Range	Average	Range	Average	Range	Average
Nitrogen	8.13 –8.44	8.32	6.26 –6.69	6.49	8.04 –8.17	8.12	7.57 –7.86	7.74
Sulphur	0.630–0.685	0.656	0.549–0.623	0.595	0.658–0.705	0.674	0.615–0.673	0.644
Phosphorus	0.692–0.702	0.698	0.706–0.728	0.720	0.639–0.669	0.659	0.681–0.700	0.693
Calcium	0.399–0.434	0.420	0.419–0.465	0.444	0.354–0.388	0.373	0.392–0.430	0.413
Magnesium	0.178–0.185	0.182	0.201–0.207	0.204	0.172–0.179	0.175	0.183–0.190	0.186
Sodium*	0.507–0.652	0.572	0.563–0.641	0.606	0.489–0.593	0.537	0.518–0.632	0.572
Potassium	1.93–2.26	2.11	2.26 –2.47	2.34	1.71 –2.17	1.92	1.96 –2.29	2.12
Calories		1600		1648		1715		1647

Average daily intake of food contains 102.9 milli-equivalents of inorganic acid and 115.1 milliequivalents of inorganic base.

Diet I was always used on Sunday, Monday, and Thursday; diet II on Tuesday and Friday; diet III on Wednesday and Saturday. At convenient times during the experiment, a complete duplicate day's diet was made up for analysis three times for each of the three diets.

* The values for sodium do not include the sodium of the extra salt used in seasoning the food. This was determined separately and varied slightly from week to week. The actual total sodium consumed during each series is given in table 3.

nitrogen lost through the skin or from the shedding of epidermis, hair, and nails, such a correction did not seem justified.

The most important data of the entire investigation are presented in figures 1 and 2. Figure 1 summarizes the average daily intake and output of calcium and phosphorus for each week and series. Figure 2 gives the values for blood calcium and phosphorus obtained at the end of each week, the actual balance for the weekly periods and for each series, and the net absorption of calcium and phosphorus. This term "net absorption" has been introduced by Harris (16) because of the impossibility of separating calcium and phosphorus which is absorbed into the body and later reëx-

creted into the large intestine from that which merely passes through the gastro-intestinal tract. The term is used in a similar manner throughout this discussion to indicate the difference between the intake of calcium and phosphorus and the fecal output. The values for phosphorus are plotted

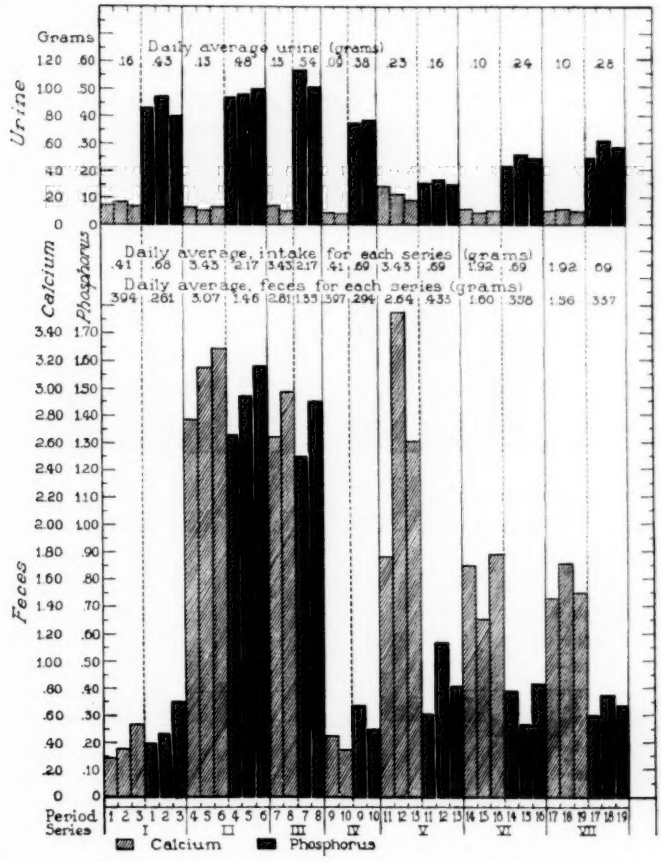
Grams
Urine
Daily average urine (grams)
.16 .43 .13 .48 .13 .54 .09 .38 .23 .16 .10 .24 .10 .28
Calcium
Phosphorus
Daily average intake for each series (grams)
.41 .68 3.45 2.17 3.45 2.17 .41 .69 3.45 .69 1.92 .69 1.92 .69
Daily average feces for each series (grams)
.394 .261 3.07 1.46 2.81 1.35 .397 .294 2.64 .435 1.60 .338 1.56 .357
Feces
Period 1 2 3 1 2 3 4 5 6 4 5 6 7 8 7 8 9 10 9 10 11 12 11 12 13 14 15 16 14 15 16 17 18 19 17 18 19
Series I II III IV V VI VII
Calcium
Phosphorus

Fig. 1. The influence of tribasic calcium phosphate, calcium lactate and viosterol on the urinary and fecal excretion of calcium and phosphorus. The numbers represent the average daily values for each series and the average daily excretion for each weekly period is represented graphically.

on a scale twice that used for calcium in order to facilitate comparison of these values in terms of the amounts of the substances necessary to form bone (approximately two to one).

During the three weeks of series I the patient showed for this entire

control period a definite negative balance of calcium and phosphorus. In each individual weekly period the negative calcium balance increased progressively and the phosphorus balance changed from a slightly positive balance to a negative balance, which also was greater the third week than

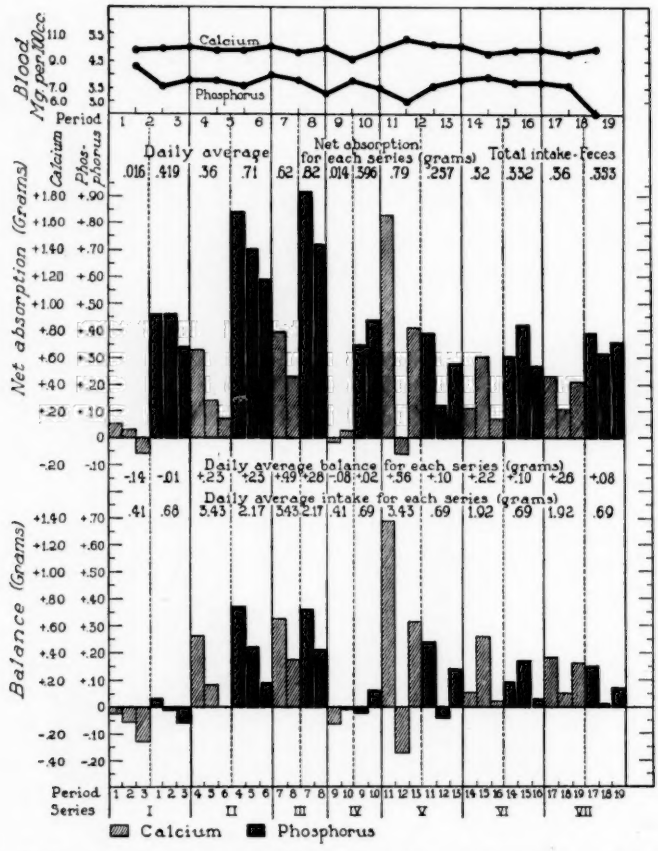
Blood Mg. per 100 cc.
Calcium
Phosphorus
Period
Daily average
Net absorption for each series (grams)
Total intake - feces
.016 .419 .36 .71 .62 .82 .014 .596 .79 .257 .52 .332 .36 .353
Net absorption (Grams)
Daily average balance for each series (grams)
-.14 -.01 +.23 +.23 +.49 +.28 -.08 +.02 +.56 +.10 +.22 +.10 +.26 +.08
Daily average intake for each series (grams)
.41 .68 3.43 2.17 3.43 2.17 .41 .69 3.43 .69 1.92 .69 1.92 .69
Balance (Grams)
Period
Series
I II III IV V VI VII
Calcium
Phosphorus

Fig. 2. The influence of tribasic calcium phosphate, calcium lactate and viosterol on the calcium and phosphorus values for the blood, the net absorption and the balance. The numbers represent the average daily values for each series and the average daily excretion for each weekly period is represented graphically.

the second. The loss of calcium was considerably greater than that of phosphorus and appeared to be greater than would normally be expected on this intake. The negative balance observed during the third week exceeded that found by Bauer, Albright and Aub for a group of normal

individuals on a much lower intake of calcium. On a daily intake of calcium of 0.11 gram these authors have reported an average negative calcium balance of 0.15 gram. Moreover, a patient with periodic paralysis who was under our observation on the same control diet as was used for this investigation was able to maintain a satisfactory calcium and phosphorus balance over a period of twenty-four days. It is also seen in figure 1 that the subject of this study was very slow in reaching calcium and phosphorus equilibrium on the control diet, although no similar delay in reaching nitrogen equilibrium was observed. The net absorption of calcium was very low, averaging only 0.016 gram daily for this entire series I, and it progressively decreased each week to such an extent that, during the third week, the calcium in the feces was 120 mgm. in excess of the intake. The net absorption of phosphorus appeared to be in the normal range. The values for blood calcium and phosphorus were also normal during this control series. The urinary excretion of calcium and phosphorus remained approximately constant throughout this series I, and the changes observed are due chiefly to variations in the fecal output. The data suggested that the chief disturbance in this subject was in calcium metabolism, apparently due to faulty absorption of calcium or to a tendency to re-excrete large amounts of calcium. It is possible that the low gastric acidity combined with the fact that the diet was slightly basic may have been a factor in the faulty absorption of calcium which was observed, although, as Schmidt and Greenberg have pointed out, solution of calcium salts by the gastric juice is not an indispensable factor in the assimilation of calcium. That a certain amount of re-excretion must also be considered is evident from the results obtained in the third week of series I. It is impossible to state how much of this latter is due to the calcium present in the intestinal secretions and how much of it may be due to a definite ability of some part of the intestine to excrete small amounts of calcium.

In the three weekly periods of series II, in addition to the calcium and phosphorus in the diet these substances were supplemented by the administration of 8 grams of tribasic calcium phosphate, containing 3.02 grams of calcium and 1.48 gram of phosphorus. This was administered in the form of an emulsion because tribasic calcium phosphate is only very slightly soluble. During series II there was a very definite retention of calcium, so that an average daily negative calcium balance of 0.14 gram was converted into a positive balance of 0.23 gram. The retention of phosphorus was nearly twice that necessary to unite with the retained calcium to form bone and this excess phosphorus was probably utilized to replenish any deficiency in the normal "floating supply" of phosphorus and to combine with the new-deposit protein formed by the retained nitrogen. The individual weekly periods of series II showed both for calcium and phosphorus, and especially for the former, a very much larger positive balance

in the first week of administration; it decreased rapidly in the second and third weeks. Part of this difference is undoubtedly attributable to the lag in obtaining equilibrium, but it seems significant that in the third week the retention of calcium was negligible, as though there was little or no tendency under these conditions for these substances to continue to be retained for formation of bone. There was no increase in the excretion of calcium in the urine but rather a slight decrease, and there was only a slight increase in the urinary excretion of phosphorus. There was also no appreciable change in the blood calcium or phosphorus. The fecal calcium and phosphorus were both high, suggesting that a large part of the tribasic calcium phosphate had merely passed unchanged through the gastro-intestinal tract. The evidence, therefore, suggests under the existing conditions that tribasic calcium phosphate, even when present in large excess, does not for more than a short time favor absorption of calcium with the formation of bone. Some factor necessary for its absorption and retention seems to be easily and rapidly exhausted. A progressively decreasing retention of calcium, produced by feeding tribasic calcium phosphate to dogs, has also been noted by Taylor, Weld, and Sykes, although these investigators do not present any actual data on this subject.

The two weekly periods of series III suggest that vitamin D may be a limiting factor in the utilization of tribasic calcium phosphate. The only difference in the experimental conditions between the periods of series II and series III was the addition of 30 minims of viosterol (10 minims, three times daily). With the addition of vitamin D in large excess there was an immediate retention of 0.65 gram of calcium daily and of approximately an equivalent amount of phosphorus (0.35 gm.) for the formation of bone. In the succeeding week the retention of calcium was not as large but was still appreciably more than the retention of calcium during the corresponding week in series II. The retention of phosphorus also decreased during the second week of this series, so that the ratio of calcium and phosphorus stored remained approximately constant. There also occurred no change in the calcium in the urine, but there was an increased net absorption, suggesting that faulty absorption had probably been an important factor and that the calcium which was finally assimilated was utilized for formation of bone. The phosphorus in the urine, on the other hand, showed a still further increase, accompanying an increased net absorption. The values for blood calcium remained constant, and because of the greater tendency for variations in the values for inorganic phosphorus in the blood, the significance of the decrease in the blood phosphate for the second week is probably questionable. It is impossible to determine from the data presented how long this combination of calcium phosphate and viosterol may be of value in producing a storage of calcium and phosphorus. Investigations of Frank and Schloss were not made over consecutive periods,

but they seem to indicate that in rickets the combination of calcium phosphate and cod liver oil may produce retention of calcium and phosphorus over an extended period.

A second control of two weekly periods on the basic diet was obtained in series IV and the results were only slightly different from those of the first control series. The calcium and phosphorus in the urine were both somewhat lower than in series I, as has been observed frequently following a relatively high intake. The net absorption was about the same, although in series IV the calcium balance was less negative in the second week than in the first. The negative balance observed immediately following a period of high intake of calcium and phosphorus might be expected before equilibrium was established on the new regimen, and the smaller loss of calcium occurring during the second week of series IV as compared to the corresponding period of series I was probably attributable to the favorable influence of the feeding of viosterol in series III immediately preceding it. There also occurred a slight drop in the blood calcium in the first week, returning to the usual value in the second week.

In the three weekly periods of series V calcium lactate (21.54 gm.) was administered in solution containing the same amount of calcium (3.02 gm.) as was contained in the tribasic calcium phosphate in series II and III. The average daily retention of calcium for this series was 0.56 gram and of phosphorus 0.10 gram. During the first week of this series the retention of calcium was very high, 1.38 gram daily, the blood calcium at the end of this week being 10.6 mgm. per 100 cc., the highest value observed throughout the investigation. The calcium excreted in the urine was definitely higher than during any of the previous series and the net absorption was the highest observed during any week. All of these facts seem to indicate that calcium lactate was much more rapidly absorbed than the insoluble calcium phosphate. This high retention of calcium was not maintained, however, although the results of the succeeding weeks differ markedly from those in the series in which calcium phosphate was administered. The fluctuating results for phosphorus suggest that the limited intake of phosphorus may be an important factor. During the first week although the retention of calcium was 1.38 gram daily, the retention of phosphorus was only 0.24 gram, an amount much too small to hold the calcium stored as bone. It was apparently possible to retain temporarily a large excess of calcium, but the negative balance occurring in the second week indicates that such a retention of calcium could not continue over a prolonged period. McGowan has also demonstrated that rabbits fed calcium carbonate were able to retain temporarily considerable calcium in excess of phosphorus. During the third week there was again a definite retention of calcium and phosphorus, with the calcium still exceeding the phosphorus, although not in as great an excess as in the first week. The net absorption of phosphorus was

definitely less for this series than during either control period, due apparently to the precipitation in the intestinal tract of a relatively large amount of phosphorus as insoluble calcium phosphate, because of the presence of the excess calcium from the calcium lactate. A decreased urinary excretion of phosphorus, however, compensated for the lowered absorption, so that an appreciable amount of the phosphorus that was absorbed was retained with the calcium for the formation of bone.

The three weekly periods of series VI were the same as the preceding series except that exactly one-half as much calcium lactate was administered. Calcium and phosphorus were both retained throughout this period, although the same type of alternating weekly values as in series V were observed. There was a slight temporary drop in the blood calcium on decreasing the intake of calcium which was similar to that observed after series IV. The decrease in the urinary excretion of calcium to a value less than that for the control series I, the continued maintenance of this low level throughout the three weeks, and the decreased net absorption of calcium, indicated the need of a large excess of calcium to produce any marked increase in the urinary excretion. It was hoped by reducing the excess calcium to some extent that a greater retention of phosphorus might occur. A greater net absorption of phosphorus was noted, but the actual average daily retention of phosphorus was the same for this series as the previous one and excess phosphorus was excreted in the urine. On the other hand, the reduction in the intake of calcium lactate decreased the net absorption and retention of calcium to such an extent that the ratio of calcium to phosphorus retained during this series was 2.2 to 1, or the ratio of calcium to phosphorus in bone.

In the three weekly periods of series VII the conditions were the same as in series VI, except that 30 minims of viosterol (10 minims, three times daily) were added to the diet. The calcium and phosphorus balances were approximately the same as during the preceding series, with relatively little change in the urinary calcium and phosphorus as well as in the net absorption of these two substances. This is quite different from the results obtained after superimposing viosterol on calcium phosphate in series III. This lack of a demonstrable effect from administering viosterol may be due to the fact that the body still retained an excess of vitamin D from that administered in series III. There was a period of eight weeks between the two series in which viosterol was administered, but it is generally recognized that the requirements for vitamin D are small and that a deficiency does not rapidly occur. The fact that the intake of phosphorus during this last series was rather low in comparison to the intake of calcium may also have limited the influence of viosterol on the retention of calcium and phosphorus.

Throughout the nine weeks on calcium lactate there was a consistent

marked fluctuation in the amounts of calcium and phosphorus retained and in the relative amounts of these substances stored, and these results suggest, as we have already mentioned, that the absorption and retention of

TABLE 3

Complete balance studies on a subject with osteoporosis

A woman, aged 65 years; height 5 feet 2½ inches, weight 88¼ pounds.

	NITROGEN	SULPHUR	CALCIUM	PHOSPHORUS	MAGNESIUM	SODIUM	POTASSIUM	
Series I								
Control								
Intake*...	7.61	0.635	0.411	0.683	0.183	2.18	2.09	
In urine*.	6.07	0.495	0.155	0.434	0.079	1.56	1.63	
Balance*..	+0.54	+0.054	−0.138	−0.012	+0.005	+0.53	+0.13	
Series II								
8 gm. $Ca_3(PO_4)_2$ daily (3.02 gm. Ca and 1.48 gm. P)								Weight: 90¾ pounds
Intake....	7.74	0.644	3.43	2.17	0.186	2.80	2.12	
In urine..	5.71	0.458	0.13	0.48	0.077	2.18	1.64	
Balance...	+1.02	+0.088	+0.23	+0.23	−0.044	+0.44	+0.06	
Series III								
8 gm. $Ca_3(PO_4)_2$ daily + 30 minims viosterol								Weight: 90 pounds
Intake....	7.74	0.644	3.43	2.17	0.186	2.37	2.12	
In urine..	6.10	0.490	0.13	0.54	0.072	1.64	1.51	
Balance...	+0.69	+0.068	+0.49	+0.28	+0.001	+0.60	+0.21	
Series IV								
Second control								Weight: 90¾ pounds
Intake....	7.74	0.644	0.413	0.693	0.186	2.67	2.12	
In urine..	6.01	0.486	0.091	0.382	0.054	2.04	1.72	
Balance...	+0.78	+0.073	−0.075	+0.017	+0.010	+0.56	+0.06	
Series V								
21.54 gm. $Ca(C_2H_3O_2)_2$ daily (3.02 gm. Ca)								Weight: 91¼ pounds
Intake....	7.74	0.644	3.43	0.693	0.186	2.28	2.12	
In urine..	5.79	0.468	0.23	0.156	0.071	1.86	1.60	
Balance...	+0.96	+0.083	+0.56	+0.104	+0.008	+0.34	+0.13	
Series VI								
10.77 gm. $Ca(C_2H_3O_2)_2$ daily (1.51 gm. Ca)								Weight: 92½ pounds
Intake....	7.74	0.644	1.92	0.693	0.186	2.26	2.12	
In urine..	5.81	0.459	0.10	0.239	0.075	1.69	1.61	
Balance...	+0.98	+0.092	+0.22	+0.096	+0.004	+0.45	+0.14	

* Grams.

TABLE 3—*Concluded*

	NITROGEN	SULPHUR	CALCIUM	PHOSPHORUS	MAGNESIUM	SODIUM	POTASSIUM	
Series VII 10.77 gm. $Ca(C_3H_5O_3)_2$ daily (1.51 gm. Ca) + 30 minims viosterol								Weight: 93¼ pounds
Intake....	7.74	0.644	1.92	0.693	0.186	2.27	2.12	
In urine..	6.11	0.463	0.10	0.278	0.076	1.64	1.64	
Balance...	+0.73	+0.101	+0.26	+0.078	+0.008	+0.54	+0.15	
Average of series I to VII daily								
Intake....	7.72	0.643	2.16	1.08	0.186	2.39	2.12	
In urine..	5.93	0.473	0.14	0.35	0.073	1.80	1.62	
Balance...	+0.82	+0.081	+0.22	+0.10	+0.005	+0.48	+0.13	

calcium was limited by the small amount of available phosphorus. It would have been interesting to have confirmed this possibility by continuing the experiment several weeks more, with the addition of food containing larger amounts of phosphate or by supplementing the food with a soluble phosphate; but it was impossible to keep the patient on the constant diet for a longer period.

The results of the whole investigation indicated that the soluble calcium lactate could be assimilated more readily than the insoluble calcium phosphate. In view of the high temporary storage of calcium that may occur in the absence of sufficient phosphorus to combine with the calcium to form bone, supplementing the lactate with a soluble phosphate fed on days when no supplementary calcium is given, may be of considerable value. With calcium gluconate Basset has obtained excellent results by supplementing it with sodium phosphate, giving both salts on the same day but not simultaneously.

Table 3 summarizes the results of all our balance studies on this patient. Throughout the investigation a definite storage of nitrogen occurred and the patient went from 88 to 93 pounds (39.9 to 42.2 kgm.). Accompanying this was a definite clinical improvement, attributable apparently to the calcium medication which produced a storage of calcium and phosphorus amounting to approximately 70 grams, expressed as tribasic calcium phosphate (bone). There appeared to be a slight but consistent increase in the average retention of nitrogen during the periods when large amounts of calcium were fed. In the individual weeks, however, there appeared to be no correlation between the changes observed in the retention of nitrogen and in that of calcium. In general, the values for sulphur paralleled the changes in nitrogen. The feeding of calcium salts appeared to have little influence on the magnesium, sodium, and potassium balances, which confirmed the results obtained by Basset after feeding calcium gluconate.

SUMMARY AND CONCLUSIONS

An extensive metabolic study lasting nineteen consecutive weeks was made on a subject who had a senescent type of osteoporosis.

Tribasic calcium phosphate, when fed in large amounts, was capable of producing a storage of calcium and phosphorus. The results of a three weeks' study on tribasic calcium phosphate seem to indicate that the extent to which this storage can be produced is limited by some factor which, as it becomes exhausted, results in a decreasing retention in each succeeding period. Viosterol added to tribasic calcium phosphate caused a still more marked retention of calcium. An insufficient supply of vitamin D may therefore be a limiting factor in the assimilation of calcium from calcium phosphate, but a more extended study is needed to determine its value over a prolonged period.

Calcium lactate caused a marked average retention of calcium during the administration of this salt which definitely exceeded the retention produced by administering an equivalent amount of calcium as tribasic calcium phosphate. The results from period to period during the administration of calcium lactate were more variable and suggested that this may be due to the limited available phosphorus.

The addition of viosterol produced little change in the calcium balance from administering calcium lactate alone, although this may be due to the fact that the viosterol added to the calcium phosphate in the preceding series continued to exert its influence throughout the investigation of calcium lactate.

The subject showed a retention of nitrogen throughout the study.

No appreciable change occurred in potassium, sodium, or magnesium balances as the result of the administration of calcium phosphate, calcium lactate, or viosterol.

Over the entire nineteen weeks, 29.3 grams of calcium and 13.3 grams of phosphorus were stored, which is in the ratio of 2.2 to 1,—the ratio of these substances in bone.

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REDUCTION OF LITTER SIZE AND MAINTENANCE OF PREGNANCY IN THE OOPHORECTOMIZED RAT: EVIDENCE CONCERNING THE ENDOCRINE RÔLE OF THE PLACENTA

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Experimental observations since the time of Fränkel, over 30 years ago, have led to a general agreement that, in common laboratory animals at least, ablation of the ovaries during pregnancy results in the untimely interruption of this condition. The list of investigators, working with forms so diverse as the cow, dog, rabbit, guinea pig, goat, spermophile, mouse, rat, and opossum, is a long one and it seems scarcely necessary to review the evidence, which has been adequately discussed elsewhere (Asdell, 1928; Hartman, 1925; Parkes, 1928).

Certain notable exceptions to the dictum that removal of the ovaries during pregnancy invariably results in abortion or in fetal reabsorption give rise to the interesting problem of why such species variations should occur. In the guinea pig, for example, Herrick (1928) and, more recently, Nelson (1934) have reported that oophorectomy may not necessarily terminate pregnancy, gestation frequently culminating in normal delivery even when the ovaries had been excised before midterm. In the mare Cole *et al.* (1930) have observed that luteinization, considered vital for maintenance of pregnancy, does not appear before the 40th day and, further, that retrogression sets in as early as the 5th month. Even more striking, Hart and Cole (1934) have found that ablation of the ovaries of a mare on the 200th day interfered in no way with the course of gestation. Finally, the human being furnishes the most striking exception, since removal of the ovaries even in early pregnancy may exercise no deleterious influence upon the continuance of gestation (Ask-Upmark, 1926; Pratt, 1927; Waldstein, 1929).

It seemed significant to the writer that the exceptions noted have been confined to species in which either 1, pregnancies are characteristically monotocous as, for example, in the human being, or 2, litter size is frequently limited. With respect to the guinea pig, for instance, in which

[1] I am grateful to Dr. R. G. Harris, Director of the Biological Laboratory, for excellent facilities afforded during the summer of 1934.

the number of young ranges from one to four, Nelson tabulated successful pregnancies as having been confined to litters of not more than one or two. Herrick, whose paper did not include litter size, reports in a personal communication that his protocols listed one or two young only in pregnancies which went to term.[2]

The observations noted would appear to be attributable to something more than coincidence, and it seemed possible that a relation may exist between litter size and dependence upon ovarian support throughout pregnancy. A relation of such nature should lend itself to experimental test through the surgical reduction of litter size in a normally polytocous form. The work upon which the present report is based has been restricted to the rat, a regularly polytocous animal and one for which agreement is unanimous that oophorectomy will invariably terminate pregnancy (e.g., Johnson and Challans, 1930; Nelson and Haterius, 1930; Hain, 1934).

EXPERIMENTAL METHODS AND RESULTS. Adult female rats were subjected to unilateral resection of a Fallopian tube[3] to insure an initial litter-size reduction. This was followed by exactly timed mating. Subsequent procedure was varied and the animals may be conveniently classed in groups, listed in the chronological order in which the experiments were undertaken.

Destruction of fetuses by cautery. In this group, actually a preliminary series, one ovary was removed seven to eleven days *post coitum*. Laparotomy was performed, under Nembutal anesthesia, upon the 12th to the 15th day, the remaining ovary was excised and fetuses in the fertile horn in excess of one were killed by piercing directly through the uterine wall with a cautery needle. Thirty-six pregnant animals were available for this experiment, of which twenty-seven reacted to the operation by abortion or reabsorption. Nine animals, however, carried through without interruption, one from the 12th, two from the 13th, and six from the 15th day following bilateral oophorectomy. In no case was delivery accomplished and the young were recovered by Caesarean section. Of nine recoveries four were removed alive on the 23rd and 24th days, i.e., one and two days past the normal time of parturition (which occurs, on the average, at the end of the 21st day in our colony). Only two fetuses were viable, however, and were successfully reared by foster mothers. Full term development in each instance was indicated by the size and weight

[2] One animal, oophorectomized on the 44th day, delivered two living and one dead young at term.

[3] Ligation of the tube proves unsatisfactory, since the periovarial capsule becomes enormously distended with fluid, a circumstance which frequently leads to a condition of permanent oestrus and consequent sterility. Removal of the ovary, on the other hand, results in a compensatory hypertrophy which defeats the purpose of operation.

at removal, weights ranging from 3.7 to 5.5 grams being recorded. The dead fetuses recovered, while fresh, were rather badly crushed or distorted, apparently by tremendous intra-uterine pressure. Each female was sacrificed and carefully examined to verify completeness of ovarian ablation.

In this preliminary series nine out of thirty-six, or 25 per cent, carried to term, and it appeared that in the rat, contrary to universal belief, the ovaries are not invariably essential during the latter part of pregnancy, providing only one fetus is being carried. The birth mechanism, however, appears definitely to break down.

Since data obtained in this group demonstrated the possibility of fetal survival in the absence of ovarian support, it remained to be determined what factors might be involved. The most obvious structures remaining after oophorectomy which might conceivably exert an ovarian-like influence appeared to be the placentae—a view, as it developed, which has recently received support from the pertinent observations of Selye, Collip and Thomson (1935). It is to be noted that the fetuses were destroyed but not removed and, moreover, the degree of placental destruction in each instance was not observed. Accordingly, two additional series were studied, 1, in which the embryos were removed surgically, whereby placental tissues were left *in situ* with relatively little trauma, and 2, in which the placentae were included in fetal removal. These will be discussed in order.

Effect of placental retention following litter-size reduction. In this group, prepared and mated as before, laparotomy was performed on the 12th, 13th and, in one case, the 14th day. The operation entailed, as before, removal of one ovary and of fetuses in excess of one. Fetal removal was effected by means of a small incision over each gestation site through which the young was expressed, care being exercised to leave the placenta intact and *in situ*. No sutures were made in the uterine incisions. Two days later, usually upon the 14th or 15th day of gestation, the remaining ovary was removed under light ether anesthesia. Results in this group were striking, and are summarized in table 1. In the ten experimental animals in every case the remaining fetus was carried to term and was recovered alive after being arbitrarily allowed to remain *in utero* for one or two days past the expected time of parturition. Placentae, including the ones of previously removed fetuses, were found to be viable and healthy, although in some instances partial separation from the uterine wall had been effected. Following this technique, therefore, and with reasonable care, it is possible to carry the rat through to term with no ovarian tissue during at least the last third of pregnancy of 100 per cent of cases. Despite the presence of placental tissue, however, spontaneous delivery did not occur, a circumstance which necessitated recovery of the young by surgical intervention.

No attempt was made in these experiments to determine quantitatively

the amount of placental material necessary to maintain one fetus. None of the experimental animals carried less than three young at the time of laparotomy and more often the number ranged from four to seven; the number of retained placentae following operation was never less than two. It would be of interest to carry out a progressive reduction in amount of retained tissue in a series of animals, although it seems probable that a certain degree of individual variation might be encountered.

Effect of surgical removal of extra fetuses and placentae. Whether or not the excess placental tissue *per se* was responsible for maintenance of the experimentally monotocous pregnancies remained to be ascertained. A third group was utilized, subjected to precisely the same treatment as the preceding series with the exception that the "extra" placentae were

TABLE 1

Effect of placental retention following litter-size reduction and oophorectomy

RAT	LAPARO-TOMY*	OOPHOR-ECTOMY	RESULT
1	13**	15	23; live fetus, 5.5 gm.; 4 extra placentae
2	14	16	23; live fetus, 5.1 gm.; 3 extra placentae
3	13	15	22; live fetus, 4.7 gm.; 3 extra placentae
4	12	14	24; live fetus, 5.0 gm.; not viable; 2 extra placentae
5	13	15	23; live fetus, 4.9 gm.; 4 extra placentae
6	13	15	23; live fetus, 5.1 gm.; not viable; 3 placentae
7	13	15	22; live fetus, 5.0 gm.; 3 extra placentae
8	12	14	23; live fetus, not viable; 5 extra placentae, 3 of which were detached
9	13	16	23; fetus, 4.8 gm. alive, but died shortly after removal; 3 extra placentae
10	13	15	23; live fetus, 4.8 gm.; 4 extra placentae, 1 detached

* Involving removal of one ovary and fetuses in excess of one.

** The figure in each column in this and in following tables represents the day of pregnancy when operation or sacrifice was carried out.

included in the surgical removal of fetuses on the 12th or 13th day. Again the remaining ovary was extirpated on the 14th or 15th day through a small lumbo-dorsal incision. Results are summarized in table 2. It will be seen that none carried through. Autopsy of these animals on the expected date of labor revealed, in the eleven animals, eight with markedly involuted uteri which contained no indications of embryonic substance, and three with persisting placental tissue. A fourth group of females were employed as controls, subjected to the same procedure except for removal of the second ovary. One ovary left *in situ* sufficed to carry these animals through successfully, with one exception, and normal delivery occurred in each instance at the expected time (table 3).

Before attempting to evaluate the foregoing observations it should be

pointed out that a characteristic feature in all cases in which fetuses were recovered lay in the almost complete absence of extra-embryonic fluids and in the tight constriction of the uterus about the fetus. If a fetus be left *in utero* more than two days past the normal date of labor it is literally crushed to death—a fate typical of the dead fetuses in the first of our experimental series. Fetal death from this cause is not uncommon following uncomplicated bilateral oophorectomy, according to Selye, and

TABLE 2

Effect of removal of fetuses and placentae, with oophorectomy

RAT	LAPARO-TOMY	OOPHOR-ECTOMY	RESULT
15	12	15	20; placenta *in situ*, large. No fetus
16	12	14	20; uterus empty and involuted
17	12	15	21; uterus empty and involuted
18	13	15	21; uterus empty and involuted
19	12	15	21; uterus empty and involuted
20	14	16	20; term-sized placenta *in situ*; no fetus
21	13	15	20; uterus empty and involuting
22	12	15	21; uterus empty and involuted
23	13	15	22; uterus empty and involuted
24	12	16	Allowed to run to 24th day. No evidence of delivery. Atrophic uterus upon postmortem
25	13	15	22; one small placenta, unattached

TABLE 3

Effect of retention of one ovary following removal of excess fetuses and placentae

RAT	LAPAROTOMY	RESULT
31	12	Delivered one fetus 22nd day; perfectly normal
32	12	Delivered one fetus 21st day; killed by mother
33	13	Delivered one fetus 22nd day; fetus died following day
34	14	Delivered one fetus 22nd day; perfectly normal
35	15	Delivered one fetus 21st day; perfectly normal
36	14	Delivered one dead fetus 21st day
37	14	No delivery. Empty uterus at 23rd day autopsy
38	14	Delivered one fetus 22nd day; perfectly normal
41	13	Delivered one fetus 22nd day; perfectly normal
42	15	Delivered one fetus 22nd day; killed by mother

occurs within a relatively short time after operation. One fetus, it would seem, is better able to survive than are several, since death from compression is considerably postponed.

Discussion. The foregoing data demonstrate, in the first place, that ovaries are not essential during at least the last third of pregnancy in the experimentally monotocous rat, provided sufficient viable placental tissue is present. Secondly, it is notable that under the experimental conditions

employed the birth mechanism breaks down. With respect to the first observation the fact that one fetus will not survive in the absence of additional placentae would seem to vitiate the possibility that it might not need continued hormonal action for complete development. It appears, therefore, that placental tissue is capable of assuming a function generally considered peculiarly ovarian, namely, the maintenance of pregnancy. Since it is well known that this property of ovarian tissue is inherent in its progestin output it seems difficult to escape the conclusion that there is a placental origin of progestin or of a progestin-like principle. Indeed, several items of evidence exist which point toward this possibility. In the first place Selye and his collaborators (1935) have recently shown that removal of ovaries, or of ovaries and fetuses, exerts no marked effect upon the subsequent fate of retained placentae, the latter persisting in viable condition until approximately the time of normal parturition. Not only do the placentae persist, according to them, but apparently exert a definite hormonal action in the inhibition of lactation and in the promotion of a type of uterine mucosal reaction usually attributed to the influence of progestin. Newton (1935) has shown that, in the mouse, removal of fetuses on the 12th to the 15th days does not interfere with retention of the placentae until normal full term, at which time they are expelled—a process which he characterizes as "pseudo-parturition." In his experiments *post partum* oestrus supervened one or two days following uterine evacuation, regardless of whether placental expulsion was induced prematurely or allowed to take place at "term"—convincing evidence that oestrus is held in abeyance, directly or otherwise, by the presence of placental tissue. It is worthy of note also that in his experiments on lactation Nelson found that retention of placentae after fetal removal in the guinea pig inhibited lactation, a suppression which was promptly relieved upon their expulsion. It was suggested that the oestrin content of the retained placentae might have been concerned in the inhibition of lactation, although the possibility that progestin may have been a factor was not ignored. Indeed, it seems probable that the action may lie in a combination of the two.

As a working hypothesis, then, it may be supposed that in the present experiments the additional, or "extra" placentae liberated enough progestin to maintain the pregnancy of a single fetus.[4] This suggests a seeming absurdity in that one placenta presumably is not sufficient to maintain one fetus; yet, in the rat, this appears to be precisely the situation. It would seem difficult to explain this circumstance, unless one argued from the standpoint of species variation—a factor, indeed, often

[4] There is, certainly, no experimental evidence to indicate that oestrin is capable of maintaining pregnancy; on the contrary administration of this principle frequently results in abortion.

overlooked in considerations of the comparative physiology of reproduction. It must be remembered that in the rat, *under normal conditions*, the corpora lutea are functional—and essential—throughout the course of pregnancy. If, therefore, the placenta serves as an adjunct to the ovaries as a secretory mechanism, and evidence on this point seems incontrovertible, its endocrine rôle in the course of gestation in the rat cannot be so important a one as might be expected, for example, in the mare or in the human being, in which the corpora lutea retrogress or become non-essential at a relatively early age and in which, presumably, the placenta assumes more or less entirely the functional attributes of ovarian tissue. Such a view would serve to explain, in any event, the curious fact that oophorectomy in the latter forms need not terminate pregnancy, and at the same time would not be at variance with our concept concerning the importance of the luteal hormone during pregnancy; on the contrary it would aid in harmonizing what have been hitherto very puzzling and conflicting data.

In brief, progestin is essential in all mammals; in some, notably a few of the monotocous forms studied to date, the placenta is capable of preempting, in large measure at least, the elaboration or release of this principle. In this connection the writer has sought in vain for a naturally occurring monotocous pregnancy in the rat, since it would be of prime importance to determine the fate of the single fetus under such circumstances. A final answer, however, can come only from extensive work of a comparative nature.

In the failure of the birth mechanism the rat differs markedly from normally monotocous forms.[5] The reason for this is not obvious. Further experimentation on the same type of animal should prove valuable since the monotocous rat, devoid of ovarian tissue, would appear an ideal form for an experimental study of factors controlling the birth mechanism. As noted elsewhere the uterus in the absence of the ovaries has lost its resiliency, is remarkably rigid and, presumably, has become insensitive to the stimuli involved in the process of labor. Since oestrin serves as a powerful sensitizer of uterine tissue (Reynolds, 1935) it is not unlikely that deficiency of this principle is an underlying factor in failure of birth. No assumption is justified at present on the basis of experimental evidence, however, and further work is being carried out in an attempt to elucidate this point.

SUMMARY

1. In rats in which litter size has been surgically reduced to one fetus the ovaries are not essential during at least the last third of pregnancy, providing the placentae of the removed fetuses have been left *in situ*.

[5] Hain (1934), in fact, reported that oophorectomy performed as late as the expected day of delivery will delay parturition for one to two days.

2. In the absence of ovaries under these conditions, however, the birth mechanism is seriously, and perhaps permanently, impaired.

3. From data obtained the hypothesis is advanced that progestin, or a principle simulating its action, has its origin in the placenta, which serves, therefore, in an auxiliary capacity to the ovaries. It is suggested that the degree of development of this auxiliary capacity may determine, in various species, the relative freedom from dependence upon ovarian support throughout pregnancy.

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THE NATURE OF VENTRICULAR ALTERNATION RESULTING FROM REDUCED CORONARY BLOOD FLOW

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The various theories held to explain the phenomenon of alternation are: a, that all muscle elements contract in both the larger and smaller beats but to different degrees; b, that all muscle fractions contract to an equal degree but that the phasic entry of contractions is slower in the smaller beat, and c, that certain fractions throughout the ventricle fail to contract in every alternate beat. While the last view is generally favored, its establishment as a fact awaits crucial evidence of the missing contractions.

In experimental studies of coronary flow by Green, Gregg and Wiggers (1), it was necessary to reduce the blood supply to a portion of the myocardium for short intervals by intermittent clamping of a main coronary vessel. Too frequent repetition or excessive prolongation of such clampings occasionally caused an annoying ventricular alternation, as manifested in aortic pressure curves. This fortuitous phenomenon provided an opportunity for making a study of the behavior of the relatively ischemic area and of the nature of alternation obtained by this method of restricting blood flow. The results of this study, which supply evidence of the missing contractions, are presented in this paper.

PROCEDURE. Dogs anesthetized with morphine and sodium barbital were used. The chest was opened under artificial respiration, the heart was exposed and suspended in a pericardial cradle and the ramus descendens anterior was isolated. An electromagnetic clamp was arranged to occlude this vessel, usually for one-half to two-thirds of each heart cycle but occasionally for two or three cycles out of every five or six. By this method the blood supply was impaired but not completely suppressed. With such procedures alternations appeared in a fair number of hearts.

Aortic pressure was recorded by a Wiggers optical manometer inserted by way of the left subclavian artery. Myocardial contractions of the area supplied by the ramus descendens anterior were studied by attaching a myocardiograph described by Tennant and Wiggers (2) which registered optically through a Frank segment capsule.

RESULTS. Figure 1 shows one type of result found in six experiments.

Marked alternation of the left ventricle is evidenced by the aortic pressure record (A.P.); which shows little more than an incisura during the smaller beats (2, 4). The myocardiogram M shows that a typical shortening curve, such as was described by Tennant and Wiggers for the normal heart, occurs in each large beat. During the period of isometric contraction the curve shows a few vibrations only but with the beginning of ejection the myocardiogram rises, indicating a shortening of the muscle. This rise extends beyond the end of systole, and the curve falls when the ventricle fills following the interval of isometric relaxation.

In the small beats no such shortening occurs during ejection; the myocardiograph line remains horizontal. This indicates that this area did not contribute toward ejection, but that a sufficient contractile effort was made to prevent stretching of the area by the increasing intraventricular tension. Such experiments show that impairment of blood supply can produce periodic variations in the degree of myocardial contraction.

In three other experiments, however, the records showed alternate shortening and lengthening during the larger and smaller beats of an alternans couple. A typical example is shown in figure 2. As denoted by arrows the myocardiogram rises in a typical manner during ejection, in the larger beats; but it descends with the onset of isometric contraction and rises promptly at the time of the incisura in the smaller beats. This indicates that the areas do not contract but extend during systole in the smaller beats. Since there is every reason to believe that the major part of the myocardium having an unimpaired blood supply contracted normally, such alternate missing of contractions in an ischemic area is undoubtedly responsible for the alternating pulse shown in the aortic pressure record.

In two other experiments, alternation was observed when contractions in the region with reduced blood supply were almost entirely suppressed. During the small beat the myocardiograph records a pronounced extension (downward movement) during the whole systole; in fact the curve resembles an inverted intraventricular pressure curve. In the larger beat, the myocardiogram shows a sharp drop during isometric contraction followed by a brief rise indicating an abortive attempt at shortening. This lasts for only a portion of systole when further extension occurs. Such curves demonstrate not only that contractions fail in the smaller beats but also that less vigorous contractions may be present during the larger beat of an alternans couple.

Discussion. The importance of these observations consists in the demonstrations 1, that reduction of coronary blood supply can lead to alternation, and 2, that alternate diminution or failure of contraction limited to a territory supplied by one coronary branch can produce alternations of ejection (pulsus alternans) that cannot be distinguished from alternations of beat involving the entire musculature of the left ventricle.

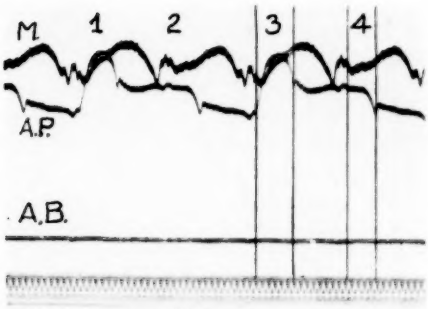

FIG. 1

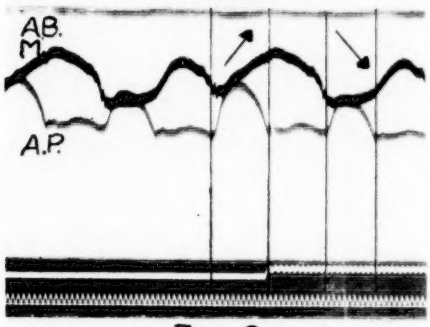

FIG. 2

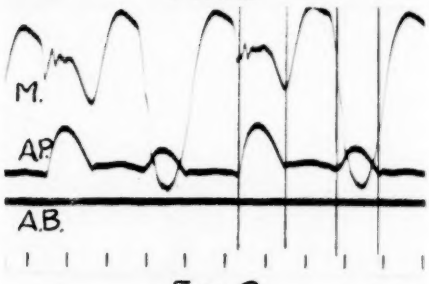

FIG. 3

Figs. 1, 2 and 3. Records illustrating the types of contractions occurring in a relatively ischemic area of the heart during ventricular alternation. *M*, myocardiograph record, downward movement indicates extension—upward movement shortening; *A.P.*, aortic pressure record; *A.B.*, base line for aortic pressure. Time, 0.02 second in figures 1 and 2, and 0.2 second between black lines at bottom of record in figure 3. (DD-28/1a; DD-32/33; C-636/5d.)

While the latter type undoubtedly occurs both experimentally and clinically, the possibility of such regional variations has not been ruled out in experimental studies of the alternans problem and it must certainly be considered in every clinical case. Furthermore, the demonstration, that blocks of ischemic muscle can alternately contract and fail, offers substantial support for the view that a wider distribution of small fractions of muscle evidencing alternate failure of contractions accounts for alternation involving an entire ventricle or the whole heart.

In alternation due to regional variations of contraction following impaired blood supply, the region affected may merely contract less efficiently during the small beat or it may fail to contract at all; whereas during the larger beat the area may be contracting normally or it may be depressed, but not to as great an extent as during the small beat.

Does a dynamic form of alternation exist? Wenckebach (3) raised the question whether all types of clinical ventricular alternations are due to fundamental myocardial derangement, i.e., whether some forms at least may not be due to alternation of ventricular filling and consequent differences in ejection. While this view has not been extensively accepted, it received some support from the observations of Wiggers (4) in the very special case of a temporary alternation following a long diastolic pause. After a thorough consideration of various dynamic possibilities he concludes that "while we may still admit the possibility that fundamental changes in cardiac muscle occur in the type of alternation discussed, experimental proof for this is lacking. On the other hand, a full consideration of experimental data shows that all the phenomena associated with alternation can be accounted for by changes in diastolic size and arterial resistance."

It remains to present experiments which seem to supply the experimental proof that a long diastolic pause can produce changes in cardiac muscle *per se* which lead to alternate contraction and failure of contraction. During three experiments, records were occasionally obtained in which a spontaneous premature contraction disturbed the rhythm and was followed by a temporary pulsus alternans. Figure 4 shows an example. A normal beat (1) is followed by a premature beat (2) which failed to open the aortic valves. This is followed by a large post compensatory beat (3) and this in turn by a small beat (4). Alternate large and small beats follow, the difference in size becoming less as in instances analyzed by Wiggers.

A study of the myocardiograph record contains many interesting features. In the first beat, the area was definitely stretched during systole, although the slight rise during the ejection period indicates that some contraction tending to resist stretching occurred. On the contrary, the rounded bottom of the second or premature beat suggests that the muscle made no attempt to shorten. In the third beat—following a long pause—the myocardiogram gives distinct evidence by its rise that the muscle was

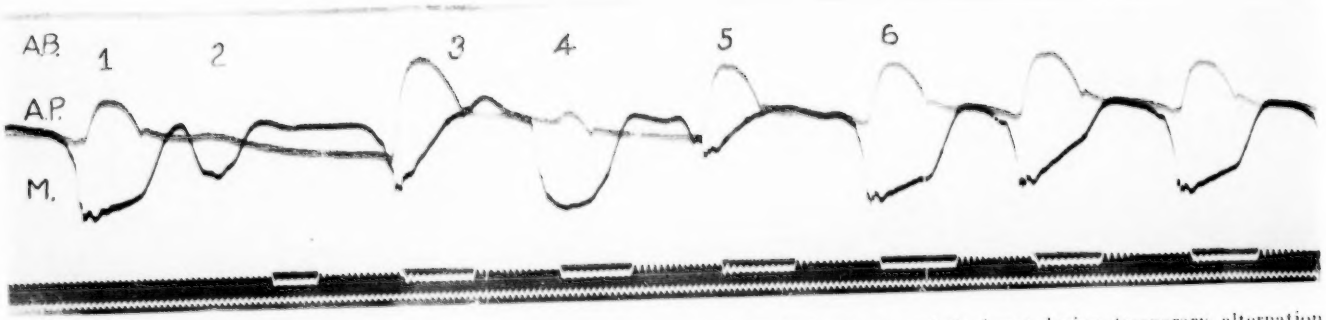

Fig. 4. Record illustrating the type of contraction occurring in a relatively ischemic area of the heart during temporary alternation following the prolonged diastole resulting from the premature systole—beat 2. Lettering and time same as figure 1 (DD-33,78).

contracting more vigorously. Since the degree of stretch occasioned in beat 2 equalled that found in beat 3, and in beat 4, certainly exceeded that found in beats 3 or 5, it is difficult to assign the recovery of contraction to changes in muscle length. These facts therefore suggest that the recuperative effect of the longer pause must be the more important. During the alternating beats that follow (5–6. . . . .) the myocardiogram shows alternate contraction and extension and this occurs despite the fact that the stretch is greater in the smaller beats. Therefore there is no possible conclusion other than that, in this type of alternation also, inherent changes in the muscle occur which lead to contractions in alternate beats only. The relationship to changes in initial tension and length found by Wiggers are probably fortuitous and in no sense to be regarded as fundamental determinants of alternations.

In view of these observations, it is very questionable whether any form of alternation (clinical or experimental) can occur without an actual failure of contractions, during the smaller beats in at least a part of a chamber manifesting such alternation.

SUMMARY

1. Ventricular alternation (pulsus alternans) was produced by periodic deprivation of blood flow to the region of the left ventricle supplied by the ramus descendens anterior, and the changes in contraction of this region were studied by a myocardiographic method.

2. The pulsus alternans displayed in optical records of aortic pressure was indistinguishable from that due to alternating beats obtained by any other procedure which presumably affects an entire left ventricle.

3. A study of myographic records showed that the region of impaired blood supply may merely contract less efficiently during the smaller beat or it may fail to contract at all; whereas in the larger beat it may contract normally or it may be depressed but not to as great an extent as in the small beat.

4. Those experiments which presented myographic evidence that contractions alternately occurred and failed in larger regions of ischemic muscles offer substantial support for the view that similar omission of contractions in more distributed fractions accounts for alternation affecting an entire ventricle or the whole heart.

5. The latter experiments suggest that the possibility of such regional alternation must be considered in experimental studies of the alternans problem and certainly in every clinical case.

6. Since alternate shortening and extension also accompanied large and small beats initiated by a sudden long diastolic pause and since the degree of stretch was greater in the small beats, it follows that the time required for recuperation, rather than dynamic changes in diastolic size or tension,

is the factor dominantly concerned in evoking such temporary alternation. It is questionable whether any form of alternation can occur without periodic diminution or absence of contraction in some fractions of ventricular muscle.

In conclusion the author wishes to take this opportunity to thank Prof. Carl J. Wiggers for his generous guidance and help.

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ON THE MECHANISM OF THE HEMATOPOIETIC ACTION OF COBALT[1]

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That cobalt produces a marked polycythemia when administered in small amounts to rats (Brand and Stucky, 1933; Myers, Beard and Barnes, 1931; Orten, Underhill, Mugrage and Lewis, 1932; Stare and Elvehjem, 1932; Waltner and Waltner, 1929), dogs (Mascherpa, 1930), mice, guinea pigs and frogs (Sutter, 1934), rabbits (Kleinberg, 1934), and pigs (Stare and Elvehjem, 1932) has been adequately demonstrated. Further investigations on rats (Orten, Underhill, Mugrage and Lewis, 1933), have shown that there is an actual increase in the number of circulating erythrocytes since the total blood volume and hematocrit are increased while the plasma volume remains essentially unaltered. Apparently the effect of cobalt is chiefly upon erythropoietic tissue since little or no permanent alteration in the total and differential leucocyte counts has been observed (Waltner and Waltner, 1929; Orten, Underhill, Mugrage and Lewis, 1932).

Little information is available at the present time regarding the mechanism involved in the production of polycythemia by cobalt. Preliminary work in this laboratory (Orten, 1935), has indicated that the spleen does not play an essential rôle in the phenomenon. It is possible that an alteration in the rate of blood formation or in that of blood destruction may be fundamentally involved. Cobalt may act directly or indirectly on the bone marrow to stimulate the production of increased numbers of erythrocytes or this element may in some manner decrease the rate of destruction of the cells and thus favor a passive accumulation of erythrocytes. If the action of cobalt is one of marrow stimulation, the administration of this element should be followed by an increase in the proportion of reticulocytes in the peripheral blood. Furthermore, the type of the reticulocyte response obtained should afford some information concerning the nature

[1] Preliminary reports were made before the Division of Biological Chemistry of the American Chemical Society at the Chicago Meeting, September, 1933, and at the New York Meeting, April, 1935.

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of the stimulus. If cobalt affects the marrow itself or some mechanism directly controlling its activity, a prompt reticulocyte increase is to be expected; if, however, the hematopoietic activity of cobalt is secondary to some slow toxic effect, a delayed response should be observed; and, finally, if the polycythemia is the result of a decrease in the rate of blood destruction, no significant change in the proportion of reticulocytes should occur. Data which appear to support the first of these hypotheses were obtained in some early investigations[3] on rats and will be described more fully in this paper. Since then similar findings on rabbits have been reported (Kleinberg, 1934).

If cobalt diminishes the rate of erythrocyte catabolism, some detectable decrease in the concentration of serum bilirubin is to be expected, inasmuch as, given a normal liver and biliary system, there appears to be a direct relationship between the amount of this pigment in the serum and the degree of blood destruction (Barron, 1931; Magath and Sheard, 1927). Hypobilirubinemia has been observed clinically in certain diseases in which there is a decreased rate of erythrocyte fragmentation (Barron, 1931).

The present investigation was undertaken in order to secure further information regarding the relative importance of increased blood formation and decreased blood destruction as factors in the production of polycythemia by cobalt. Changes in the proportion of reticulocytes subsequent to cobalt administration were followed both in young rats, in which blood changes presumably may be more easily elicited, and in adult rats which are probably more "hematologically stable." The concentration of serum bilirubin was also determined in the latter animals.

EXPERIMENTAL. Male albino rats of the Connecticut Agricultural Experiment Station strain were used throughout these experiments. All of the animals were kept in individual wire cages and were cared for in the manner previously described (Smith, Cowgill and Croll, 1925). In the first group of experiments, weanling rats weighing from 40 to 50 grams were given a basal ration consisting of powdered whole milk (Klim) supplemented daily by 0.5 mgm. of iron as purified ferric chloride and 0.025 mgm. of copper as recrystallized copper sulfate. When the animals reached 40 days of age, they were divided into two groups; one (10 rats) was continued on the basal milk-iron-copper diet as controls, and the other (12 rats) was fed the basal ration supplemented by 0.5 mgm. of cobalt as purified cobaltous chloride daily. Each of the mineral supplements was measured by pipette from a stock solution into about 10 cc. of a thick suspension of the dry milk in redistilled water. A supply of the powdered milk and redistilled water was available to the animals at all times.

In the experiments on adult rats, animals weighing from 250 to 330 grams

[3] Reported at the Chicago Meeting of the American Chemical Society, September, 1933.

at 90 to 100 days of age were used; they were fed the stock colony ration. The experimental group (11 rats) received the same amount of cobalt as did the young rats in the first experiments; however, due to the nature of the diet, it was necessary to inject (subcutaneously) the cobalt solution in order to assure its quantitative administration. A control group of animals (8 rats) received daily injections of an equal amount of physiological saline solution.

The animals of the foregoing groups were observed during an experimental period of from 8 to 10 weeks. Body weights were recorded weekly, and the hemoglobin and erythrocyte levels were determined bi-weekly. Reticulocyte enumerations were made on alternate days during the first two weeks of the experiment and at 4 to 5 day intervals thereafter. The procedures employed for the determination of hemoglobin, erythrocytes, and reticulocytes have been described in an earlier publication (Orten and Smith, 1934). Serum bilirubin determinations were made at the end of the eight-week period on the blood of the animals which had received injections of either sodium or cobaltous chloride. The following procedure was used:

The animals were anesthetized with a freshly prepared solution of urethane (dose 0.2 gm. per 100 gm. body weight) given by stomach tube. The blood was drawn from the heart into a well-oiled 10 cc. Luer syringe and was discharged before clotting occurred into a small heavy-walled pyrex test tube which had been previously coated with mineral oil. The blood was then centrifuged in the test tube for 30 minutes at a speed of 2000 r. p. m. The resulting clear serum was removed by a long medicine dropper into a clean dry test tube and the bilirubin content was determined by a modification (Gibson and Goodrich, 1933) of the van den Bergh procedure. The only departure from this method was in the use of a "blank serum control" for each determination. The "blank" was treated in the same manner as the actual sample with the exception that the chromogenic reagent (Ehrlich's) was replaced by an equal quantity of water. The use of this step is regarded as essential in order to compensate for pigments and interfering substances present in serum which would otherwise give a high reading. The diazotized solutions were filtered through Whatman no. 40 paper and the clear filtrate was promptly read against the bilirubin standard in a micro-colorimeter, using a green filter (Zeiss L II) in the eye-piece to facilitate the matching of the usually pale colors. A standard more dilute than that described by Gibson and Goodrich was prepared by diluting one part of the "10 mgm." bilirubin[4] standard, described by these investigators, with four parts of a diluent of the same composition as that used in the preparation of the original standard. The use of the weaker "2.0 mgm. standard" undoubtedly insures greater accuracy in the matching of the samples of low bilirubin content, such as those encountered in this investigation. Repeated experiments have demonstrated that amounts of pure bilirubin as small as 10 to 20 micrograms, corresponding to a concentration of 1 to 2 mgm. per 100 cc. of serum, can be recovered from rat serum with an error no greater than 15 per cent. Somewhat greater accuracy of recovery was found when samples containing larger amounts of bilirubin, equivalent to 5 to 10 mgm. per 100 cc. of serum, were employed.

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[4] Appreciation is expressed to Dr. R. B. Gibson of the Pathological Laboratory, University Hospitals, The State University of Iowa, for a generous sample of pure bilirubin.

RESULTS. *Changes in the proportion of reticulocytes.* The changes occurring in the proportion of reticulocytes, together with those in body weight, hemoglobin and erythrocytes are summarized in the accompanying tables. The body weights of the control animals fed the milk-iron-copper diet (table 1) are only slightly lower than those of rats fed the stock colony ration (Orten and Smith, 1934). One animal of this group later attained a weight of 513 grams at an age of 240 days. The concentration of hemoglobin and number of erythrocytes in the blood of these animals likewise increased at a rate somewhat less than that found in stock rats of the same age (Orten and Smith, 1934). These values remained at a slightly subnormal level in animals which were followed longer than the period indicated in the table. It is difficult to believe that this was due to a suboptimal daily allowance of iron and copper inasmuch as the cobalt-treated animals given the same amounts of these elements attained hemoglobin and erythrocyte levels far above those of normal stock rats. Other factors, as yet unknown, must be involved. The percentage of reticulocytes in the blood of the milk-iron-copper animals decreased at a rate similar to that of young stock rats of the same age (Orten and Smith, 1934). The final reticulocyte values, however, were somewhat higher than those of comparable stock rats, as might be expected in view of the slightly subnormal levels of hemoglobin and erythrocytes.

The growth rate of the cobalt-fed animals was distinctly lower than that of the controls, as has previously been observed. The concentration of hemoglobin and number of erythrocytes rose steadily and by the end of 6 weeks attained the high values usually found in this type of polycythemia. Significant deviations in the reticulocyte values from those of the controls occurred. There was usually a slight initial reticulosis, reaching a maximum on about the fourth day. Secondary rises reaching a maximum at about the 20th to 24th day were consistently found. The average data given in table 1 do not clearly show the secondary rise since the increase occurred at different times in different rats and therefore is less distinct in the averaged data than in the individual cases. Although further transitory increases in reticulocytes occurred in some instances, the values toward the end of the experimental period differed little from those of the controls.

The data obtained on the more "hematologically stable" adult animals (table 2) show general agreement with those found in the younger rats. The body weight, hemoglobin, erythrocyte, and reticulocyte values of the saline-injected controls compare favorably with those of untreated stock rats of the same age. The cobalt-injected animals showed the typical retardation of growth and the characteristic increases in pigment and in erythrocytes. As in the case of the cobalt-fed group, a variable, slight, early reticulosis followed by the consistent secondary rise at about the 23rd

TABLE 1

Hemoglobin, erythrocytes and reticulocytes in the blood of control and cobalt-fed rats

DAYS OF EXPERIMENT*	BODY WEIGHT		HEMOGLOBIN		ERYTHROCYTES		RETICULOCYTES	
	Average	Range	Average	Range	Average	Range	Average	Range
				Control				
	gm.	*gm.*	*gm. per 100 cc.*	*gm. per 100 cc.*	*m. per c.mm.*	*m. per c.mm.*	*per cent*	*per cent*
0	120	101–150	10.6	8.3–13.2	6.5	5.5– 7.2	7.9	6.8–10.0
2							6.5	4.9– 7.7
4							6.9	3.3– 9.8
6							5.8	3.6– 7.6
8							5.2	2.0– 7.7
10							5.1	2.2– 8.6
12							4.6	2.0– 7.2
14	180	148–223	13.1	11.3–14.6	6.7	5.4– 8.1	4.4	1.4– 7.2
19							4.7	1.4– 7.0
24							3.6	1.3– 5.8
28	231	181–282	13.1	11.6–14.5	7.1	5.1– 8.6	3.7	1.1– 5.4
33							3.0	1.2– 5.5
38							3.0	0.8– 4.7
42	275	222–323	13.9	12.5–15.6	7.7	6.7– 8.8	2.8	1.3– 4.0
56	303	248–367	14.2	13.5–15.6	7.7	6.6– 8.6	2.8	0.6– 4.4
70	328	275–396	14.7	13.5–15.8	8.4	7.4– 8.9	2.5	1.0– 3.7
				Cobalt-fed				
0	117	100–130	10.5	8.1–12.3	6.3	5.1– 7.6	8.2	6.1–13.2
2							9.0	5.4–12.0
4							9.6	5.1–17.1
6							7.6	3.3–12.2
8							6.6	3.1–10.8
10							6.4	3.2–10.8
12							5.8	1.8–11.4
14	168	133–195	14.4	11.4–16.3	7.1	4.9– 8.3	5.8	2.3–11.2
19							5.5	2.1– 9.3
24							5.9	2.2– 9.4
28	206	159–245	17.3	16.3–18.9	8.4	7.5–10.1	4.0	1.1– 6.7
33							3.2	0.9– 4.9
38							3.4	1.2– 5.2
42	222	178–252	18.5	15.9–21.6	9.8	8.1–11.3	3.0	1.1– 5.3
56	246	189–283	18.7	16.4–21.2	9.7	8.5–10.6	2.2	1.6– 2.8
70	255	201–302	20.2	16.6–24.2	10.2	8.5–12.3	3.1	1.6– 5.2

* The ages of the animals in days may be obtained by adding 40 to the figures in this column.

day was observed. Subsequently the reticulocyte level decreased to normal values.

Changes in serum bilirubin. The concentration of bilirubin in the sera

TABLE 2

Hemoglobin, erythrocytes and reticulocytes in the blood of control and cobalt-injected rats

DAYS OF EXPERIMENT*	BODY WEIGHT		HEMOGLOBIN		ERYTHROCYTES		RETICULOCYTES	
	Average	Range	Average	Range	Average	Range	Average	Range
	Control							
	gm.	*gm.*	*gm. per 100 cc.*	*gm. per 100 cc.*	*m. per c.mm.*	*m. per c.mm.*	*per cent*	*per cent*
0	278	250–331	16.6	13.5–20.5	8.1	6.1– 9.2	2.2	0.5–3.9
4							1.8	0.9–2.8
6							1.8	1.1–2.4
8							1.8	1.1–3.9
10							1.6	1.1–2.4
14	298	249–368	16.9	15.3–18.0	8.1	6.5– 9.6	1.5	1.2–2.5
18							1.3	0.7–1.8
23							1.5	0.9–2.5
28	309	261–391	17.4	16.6–19.0	8.6	8.0– 9.4	1.5	0.8–1.9
32							1.2	0.9–1.6
37							1.4	1.0–2.1
42	328	271–414	17.9	16.5–19.2	8.6	7.7– 9.2	1.3	0.9–2.2
56	345	281–444	17.0	14.4–18.7	8.4	7.0– 9.5	1.5	0.9–2.8
	Cobalt-injected							
0	280	262–301	16.8	14.8–17.9	8.2	6.2– 9.3	2.2	1.6–2.9
4							2.3	1.0–4.2
6							2.1	0.8–3.4
8							1.9	1.2–4.2
10							2.1	1.6–3.2
14	278	263–312	20.0	16.6–22.4	9.2	8.3–10.1	2.4	1.7–3.5
18							2.3	1.6–3.5
23							3.0	1.9–6.8
28	277	251–310	24.0	22.0–25.9	10.1	8.9–11.2	2.8	1.3–4.0
32							2.8	1.4–3.7
37							2.7	1.6–5.2
42	279	257–298	26.1	23.6–28.4	10.7	8.8–14.3	1.6	1.0–2.5
56	281	242–302	25.3	22.7–27.5	10.9	9.5–12.3	1.7	1.0–2.8

* The approximate ages of the animals in days may be obtained by adding 100 to the figures in this column.

TABLE 3

Serum bilirubin of control and cobalt-injected rats

GROUP	NUMBER OF RATS	TOTAL		BLANK		SERUM BILIRUBIN	
		Average	Range	Average	Range	Average	Range
		mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
Control	8	1.0	0.6–1.4	0.6	0.4–0.8	0.5	0.3–0.7
Cobalt-injected	12	2.9	2.0–3.9	1.9	1.1–2.7	1.0	0.7–1.3

of the control and cobalt-injected rats are given in table 3. The values for the control "saline-injected" animals agree well with those which have been obtained on normal rats of this colony consuming either the stock ration or certain other adequate synthetic diets (unpublished data). It is of interest to note that these values are slightly less than those obtained by other investigators on rats (Giannini, 1929) and on man (Barron, 1931; Cantarow, 1934; Gibson and Goodrich, 1933). The bilirubin content of the serum of the cobalt-treated animals, however, was distinctly higher than that of the controls. Not only was the "total chromogenic material" present in greater amounts but also the level of substances responsible for the blank reading, chiefly perhaps extracellular hemoglobin itself, was elevated. The increased blank reading was not unexpected inasmuch as the sera of the cobalt-treated animals was, without exception, a bright red color in contrast to the nearly colorless sera obtained from the control animals by the same procedure.

Discussion. The foregoing observations that the administration of cobalt elicits increases in the proportion of reticulocytes in the blood and increases somewhat, rather than decreases, the concentration of bilirubin in the serum, indicate that the hematopoietic action of this element is one of active stimulation rather than one of enhancing a passive accumulation of erythrocytes. The nature of the stimulus, whether direct or indirect, is not clearly revealed by these data because of the apparent lack of consistency in the early reticulocyte rise. It is possible that this inconsistency may be due to the fact that the time interval between determinations was necessarily too long to permit the detection of the early transitory rises in every instance.

The question of the significance of increases in reticulocytes of no greater magnitude than certain of those described may be raised. It should be borne in mind, however, that the control animals did not show such rises, and furthermore, that the animals under investigation are normal and that there is no demand for erythrocytes other than that imposed by cobalt itself. The current view on the latter point is that, given a normal bone marrow and sufficient hematogenic material, the height of the reticulocyte rise from an adequate stimulus is inversely proportional to the concentration of hemoglobin and erythrocytes in the blood at the time the stimulus is applied. Therefore reticulocyte responses of the type observed are significant beyond question.

The occurrence of bright red sera in all of the animals receiving cobalt is of particular interest. It is possible that this is merely a mechanical effect, that is, an increased rate of fragmentation as a result of the increased numbers of erythrocytes present in the blood. Or it may be that cobalt exerts some hemolytic activity under the conditions of this experiment, although the available evidence from *in vitro* experiments does not indicate that the

cobalt ion (as cobalt nitrate) possesses such a property (Hooker, 1930). However, if this were true *in vivo*, an explanation of the hematopoietic activity of cobalt might be made as follows: That the accumulation of a sufficient quantity of cobalt in the organism would produce hemolysis thus eventually increasing the bilirubin content of the serum, as was actually found; the increased amount of bilirubin might then act as the ultimate hematopoietic stimulant as has been recently suggested (Verzár, Árvay, Peter and Scholderer, 1933). The present data, however, do not warrant the statement that the increase of bilirubin is a cause rather than a result.

Another suggestion may be advanced to explain the action of cobalt in producing polycythemia. It has been demonstrated that this element produces a marked vasodilatation when administered either to rabbits or to man (Le Goff, 1934). The suggestion has recently been made (Reznikoff, Foot and Bethea, 1935) that an etiological factor in polycythemia vera may be the vascular changes in the capillaries, particularly of the bone marrow, thus producing a local anoxemia which, as is well known, favors increased blood formation. It is possible that the daily administration of cobalt causes a continuous dilatation of the smaller blood vessels and capillaries and thus produces the blood changes observed.

SUMMARY

The oral and the subcutaneous administration of cobalt to young and to adult albino rats, respectively, produces significant increases in the proportion of reticulocytes in the blood preceding the typical elevation in the concentration of pigment and erythrocytes.

The concentration of bilirubin in the serum of cobalt-injected adult rats is slightly greater than that of comparable control animals.

These results indicate that the hematopoietic activity of cobalt is a result of an increase in the rate of formation of hemoglobin and erythrocytes and that a passive accumulation of red cells as a result of a diminished rate of cell destruction does not occur.

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THE CORNEO-RETINAL POTENTIAL DIFFERENCE AS THE BASIS OF THE GALVANOMETRIC METHOD OF RECORDING EYE MOVEMENTS[1]

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A new and unique method of recording eye movements galvanometrically has recently been developed and independently described by Dr. I. Leon Meyers (1929) and Dr. Edmund Jacobson (1930). These writers state that if suitable electrodes are placed on the temples and are connected with a sensitive string galvanometer, movement of the eyes to the right causes a deflection of the string in one direction and movement of the eyes to the left causes a deflection in the opposite direction. Under these conditions vertical eye movements have little or no galvanometric effect. If, however, the electrodes are placed one directly above and the other directly below either of the two eyes, upward movement of the eyes causes the string to be deflected in one direction and downward movement causes it to be deflected in the opposite direction, whereas horizontal movements are now ineffective. Stated more generally, a galvanometric deflection is produced when the eyes are moved in the same plane as the electrodes but not when they are moved in the plane at right angles to the electrodes. The galvanometric effect increases *pari passu* with the eye movement and persists as long as a given position of the eyes is maintained.

Although we have been able to confirm in every detail the descriptive statements made by Meyers and Jacobson concerning the relationship between the direction of eye movements and the resulting galvanometric effects, we feel that their explanation of these effects as due to the summation of action currents from the extrinsic ocular muscles is highly untenable. So far as we have been able to ascertain from the literature on the electro-physiology of muscle, there is no evidence that action currents from a contracted intact muscle ever summate in such a manner as to produce a potential difference between the muscle as a whole and its (more or less relaxed) antagonist or the surrounding tissue. Moreover, if such summation did occur, the resulting galvanometric deflection would scarcely be expected to be as smoothly sustained, i.e., free from the jaggedness char-

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acteristic of true electro-myograms, as are the deflections shown in the records published by Meyers and Jacobson (especially those published by Meyers).

Since both Meyers and Jacobson used as their recording instrument the string galvanometer, they presumably employed an exosomatic source of current for balancing the string. If this was true, the galvanometer could be deflected not only by actual potential variations between the electrodes but also by resistance changes. That the galvanometric effects which these writers noted were due merely to resistance changes seems very unlikely, however, in view of the fact that movement of the eyes away from their median position in one direction causes the galvanometer to be deflected one way and movement of the eyes in the other direction causes it to be deflected in the opposite manner. If movement of the eyes away from the median position to the right produced, let us say, a decrease in resistance between the two electrodes, a comparable movement away from the median position to the left should likewise produce a decrease in the resistance and therefore a deflection of the galvanometer in the *same* direction as in the former case, which is contrary to the observed facts.

It seemed to the present writers that the only plausible remaining explanation of the results reported by Meyers and Jacobson is that a persistent potential difference exists between the back and the front of the eye and that the galvanometric effects associated with eye movements are due to change in the pattern of the electrical field which this potential difference imposes upon the neighboring anatomical structures. Certain deductions flow from this hypothesis which are diametrically opposed to those which derive from the action-current hypothesis suggested by Meyers and Jacobson. By experimentally testing these deductions, it has been possible to show which of the explanations is the valid one.

RESULTS. *The character and polarity of the galvanometric effects.* For reasons already indicated, it seemed quite improbable that the galvanometric effects which Meyers and Jacobson have shown to be associated with eye movements are due to resistance changes in the living tissues between the two electrodes. Nevertheless, it was desirable to obtain, at the outset, direct evidence on this point. In order to do this, specially designed non-polarizable electrodes[2] were connected with the in-put of a single stage direct-current vacuum-tube amplifier, the out-put of which went to a galvanometer of the ballistic type. By means of a variable compensating potential in the plate circuit of the amplifier, which was shunted across the leads to the galvanometer, it was possible to keep the

[2] We are indebted to Dr. L. F. Nims of the Department of Physiology, Yale University School of Medicine, for designing and constructing these electrodes. The electrodes proper were made of silver-silver-chloride and were immersed in isotonic saline which provided contact with the subject's skin.

galvanometer balanced without impressing any current whatever on the grid circuit, i.e., across the electrodes. This arrangement rendered the current flowing in the plate circuit so independent of resistance changes in the grid circuit that the latter could be completely shorted or completely opened without producing a detectable movement of the galvanometer, although a potential difference of only one or two microvolts between the electrodes was sufficient to give the instrument a decided throw.

With the apparatus just described it was found that eye movements give rise to galvanometric effects which are strictly comparable to those obtained when the electrodes are connected directly to a string galvanometer. This finding decisively demonstrates that these effects are due, not to resistance changes between the electrodes, but to true potential variation. (This variation is of the order of 0.50 to 0.75 millivolt for movement of the eyes from the median position to an extreme lateral or vertical position.)

If the galvanometric effects associated with eye movement be due to the summation of muscle action currents, as Meyers and Jacobson suggest, then a contracted (metabolically more active) eye muscle should presumably be negative with respect to a more relaxed eye muscle. Thus, when the eyes are turned to the right, the right temple, being in the vicinity of the external (contracted) rectus of the right eye, should be negative with respect to the left temple, which is near the external (relatively relaxed) rectus of the left eye. On the other hand, if the galvanometric effects associated with eye movement be due to a potential difference between the retinal and corneal halves of the eye, exactly the opposite relations should prevail. Thus, when the eyes are directed to the right, the left temple, by virtue of the nearness of the metabolically more active (and therefore presumably negative) retina of the left eye, should be negative, and the right temple should be positive.

Extensive observations, with both human and animal subjects, have shown that when an electrode is placed on each of the two temples, the polarity of the galvanometric effects occurring with horizontal eye movement is uniformly and invariably consistent with the expectation arising from the corneo-retinal potential-difference hypothesis, i.e., the left temple is always negative when the eyes are directed to the right and the right temple is always negative when the eyes are directed to the left. Similarly, when the electrodes are placed one above and one below either of the two eyes, the lower electrode becomes negative when the eye movement is upward and the upper electrode becomes negative when the eye movement is downward. The action-current hypothesis would demand that the polarity of these galvanometric effects be the reverse.

Passive movement of the eyes. If the electrical effects accompanying eye movement originate in the extrinsic ocular muscles, these effects should fail to appear when the eyes are moved passively. On the other hand, if

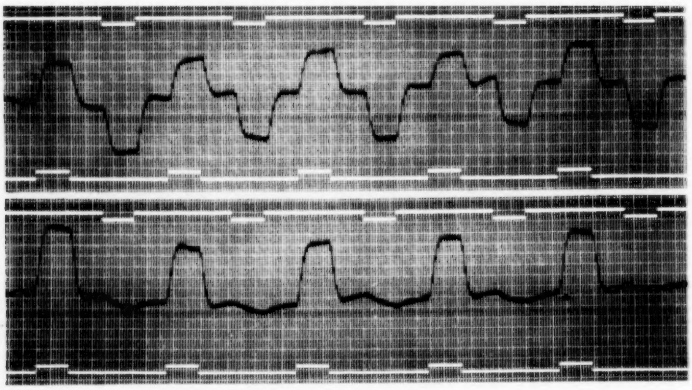

Fig. 1. Photographic records of the galvanometric deflections produced by passively moving the eyes of an anesthetized cat.

In the upper record, first the right eye was moved from the median position inward as far as possible and then, after an interval of 12 seconds, was returned to its original position. Twelve seconds later, the left eye was similarly moved from the median position inward and then, after another interval of 12 seconds, was returned to its original position. This alternation of inward movements of the right and the left eye was continued until each eye had been moved five times. The elevations in the lower (white) signal line indicate the periods during which the right eye was displaced; the depressions in the upper signal line indicate the periods during which the left eye was displaced. It will be noted that the galvanometric deflections (black line) thus produced by the independent inward movement of the two eyes are in opposite directions. These deflections follow the same general pattern as do those which are obtained when a human subject voluntarily moves *both* eyes conjugately from the center to the left, then back to center, from the center to the right, then back to center, etc.

After the upper record was obtained, 0.15 cc. of a 5 per cent solution of chromic acid was injected into the posterior chamber of the *left* eye. Within a few seconds after the injection, the pupillary and lid reflexes to light disappeared and there was a noticeable diminution in the galvanometric effect produced by moving the eye inward, as may be seen from the lower record in the above illustration. Movement of the right (uninjected) eye continued to cause a normal galvanometric deflection.

In obtaining both of the records reproduced above, the electrodes (silver dimes) were located on shaved areas just posterior to the external canthus of each eye. The cat was kept under surgical anesthesia (both local and general), and the electrodes were left in position throughout the experiment. The records read from left to right. The heavy vertical lines (separated by four fine lines) represent intervals of five seconds. The heavy horizontal lines (likewise separated by four fine lines) were one centimeter apart in the original records.

these electrical effects arise from a corneo-retinal potential difference, they should occur with passive and active movements alike. Records obtained from cats under full sodium amytal anesthetic, supplemented by narco-

tization of the cornea and conjunctiva with 2.0 per cent novocain, show that passive eye movements (produced by means of a probe caught in the cornea) give rise to galvanometric effects which are virtually identical with those obtained in human subjects during voluntary eye movements (see the upper record in fig. 1).

In order to anticipate the objection that the records obtained from passive eye movements in the cat might be due to action currents produced by the proprioceptive stimulation of the ocular muscles through stretching, the experiment described immediately below was performed.

Experimental destruction of the retina. If the explanation advanced by Meyers and Jacobson for the galvanometric effects associated with eye movement be correct, careful destruction of the retina (by chemical means) should not alter these effects. However, if our hypothesis of a corneo-retinal potential difference as the cause of these effects be valid, destruction of the retina would be expected seriously to diminish or perhaps entirely eliminate the galvanometric consequences of eye movement. In order to determine which of these two opposed inferences is true, we performed the following simple experiment on several deeply anesthetized cats. Electrodes (silver dimes) were placed on shaved areas just lateral to the external canthus of each eye. After photographic records had been obtained of the galvanometric deflections produced by passive lateral movements of each eye, 0.15 cc. of a 5 per cent solution of chromic acid was injected into the posterior chamber of one of the eyes. Immediately there set in a progressive diminution in the galvanometric consequences of passively moving the injected eye. At the end of an hour, this process had progressed to the point where movement of the injected eye produced practically no galvanometric effect, although the uninjected eye continued to show the usual electrical phenomena. Typical results of this experiment are shown in figure 1. When the animals were tested 24–48 hours after injection, passive movements of the injected eye likewise failed to produce any significant galvanometric effect.

Direct evidence of a corneo-retinal potential difference. Following the experiments described above, we explored with non-polarizable silver-silver-chloride electrodes both the freshly excised and the exposed but intact eye of the turtle. We found that our supposition is correct: there is a definite potential difference between the front and the back of the eye, the latter being consistenly negative with respect to the former. Moreover, we have since discovered abundant corroborative evidence on this point in the writings of other investigators. This evidence has been excellently reviewed by Kohlrausch (1931) and will not be elaborated here.

SUMMARY

That the galvanometric method of recording eye movements recently reported by Meyers and by Jacobson is based, not upon the summation of

action currents from the extrinsic ocular muscles, but upon a persistent corneo-retinal potential difference, presumably arising from the high metabolism of the retina and the (relatively) low metabolism of the cornea, is indicated by the following facts:

1. The observed polarity of the galvanometric effects associated with eye movements fulfills the expectations arising from the corneo-retinal potential difference hypothesis and not those from the action-current hypothesis.

2. Passive movements of the eyes produce galvanometric deflections strictly comparable in magnitude and polarity to those produced by active, voluntary eye movements.

3. Movements of the eyes are accompanied by virtually no galvanometric effect after the retina is destroyed by chemical means.

4. The existence of a corneo-retinal potential difference has been directly demonstrated by earlier investigators as well as by our own observations.

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VITAMIN G CONCENTRATES AS PREVENTIVES AGAINST BLACK-TONGUE

With Parallel Studies of the Same Concentrates in the Diets of White Rats

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In 1927 Underhill and Mendel (1) described an extensive series of experiments dealing with a dietary deficiency disease in dogs which had previously been described as pellagra-like by Chittenden and Underhill (2). The morbid anatomy of this canine disease was studied by Lambert and a report of his findings is included in the report of Underhill and Mendel.

The morbid anatomy, as described by Lambert, agrees in the main so completely with the later report of the pathology of experimental black-tongue by Lillie (3) that there would seem to be little question that both were studying the identical canine disease.

Goldberger and his associates, having been engaged in an extended investigation of the etiology of pellagra, had come to the conclusion that black-tongue in dogs was the analogue of pellagra in the human. This conclusion has been further substantiated by a comparative study of the tissue changes in experimental black-tongue of dogs and in pellagra as reported by Denton (4). A wide variety of natural food materials was investigated (5), under the auspices of the U. S. Public Health Service in continuation of the researches begun by Goldberger, for their relative richness in this pellagra-preventing or "P-P factor."

Those foods which prevented pellagra were also effective for the prevention of experimental black-tongue. With the rapidly accumulating knowledge of the multiple nature of the "B-vitamins" and their respective distributions in natural foods, there was considerable indication, at least, that the "P-P factor" and vitamin G were identical.

The vitamin G concentrate used in the investigation reported here for tests of black-tongue prevention and for parallel studies of growth-promotion in white rats carried both vitamin G (lactoflavin) and the "new heat-stable" factor or factors of the vitamin B-complex. The vitamin concentrate was prepared by a preliminary extraction of low-lactose whey powder with boiling 95 per cent ethyl alcohol, followed by concentration and drying of this extract and a reëxtraction of the dried alcohol extract with a mixture of chloroform and alcohol followed by concentration and

drying. The details of the preparation have been described in an earlier report (6). This concentrate when supplied to young rats at a level of 25 to 35 mgm. per rat per day with additional supplements of crystalline vitamin B (B_1) as the only sources of factors of the vitamin B-complex induced a steady growth rate of 7 to 8 grams per week.

The seven dogs used in control and black-tongue preventive tests were male dogs ranging from 6 to 10 kgm. in body weight and were of mongrel types especially selected for unpigmented mucosa of the mouths. The dogs had every appearance of being in excellent physical condition at the beginning of the experiment. Dogs 1, 2 and 3 were given a conditioning diet (Sebrell (7) diet 326 as recommended by him) for four weeks before starting them on the experimental diet as an added factor of safety but this preliminary period was abandoned in the case of the other four dogs. During the course of the experiments the dogs were weighed weekly. Rectal temperatures were taken and examinations for lesions of the mouth were made daily. The practice of coprophagy was eliminated in all cases.

Each of the dogs was given one of two black-tongue producing diets which were prepared essentially as recommended by Goldberger and his co-workers (8, 9) and which have been designated by them with laboratory numbers 123 (after some modification no. 268) and 324 respectively. We used a complete salt mixture (Osborne and Mendel salt-mixture with added copper sulfate) in each of the diets and included more cod liver oil in diet 324, neither of which modifications appears to have made any change in the production of black-tongue. The compositions of these black-tongue producing diets as used by us are as follows:

Diet 123[1]

	grams
White cornmeal	389
Cowpeas (California black-eyed)	50
Casein (extracted with 60% alcohol)	60
Sucrose	32
Cottonseed oil	30
Cod-liver oil	15
Osborne and Mendel salt-mixture	24
	600

Diet 324[1]

	grams
White cornmeal	310
Casein (extracted with 60% alcohol)	150
Sucrose	72
Cottonseed oil	26
Cod-liver oil	31
Osborne and Mendel salt-mixture	24
	613

[1] These diets are essentially those used by Goldberger with slight modifications as explained in the text.

Each of these dietary formulas represents approximately a 2400 calorie quantity. Diet 123 contains 16 per cent and diet 324, 26 per cent of protein.

The control dogs were fed daily quantities of one or the other of these diets equivalent to 100 calories per kilo of body weight. The allowances for the dogs receiving the vitamin concentrate were limited to 80 calories per kilo. The cornmeal and cowpeas used in these diets were purchased in single lots in sufficient quantities to last throughout the duration of the experiments.

Two control dogs (dog 1 and dog 2) which received diet 123 showed definite prodromal symptoms involving the nervous system and gastro-intestinal tract after 19 and 18 days respectively, unmistakable mouth lesions after 33 days in each case and died after 37 and 38 days respectively.

TABLE 1

Summary of control dogs on black-tongue producing diets

	DOG 1	DOG 2	DOG 6	DOG 7
Diet no	123	123	324	324
Prodromal symptoms became definite	19th day	18th day	16th day	17th day
Mouth lesions developed	33rd day	33rd day	36th day	36th day
Death occurred	37th day	38th day	56th day	38th* day
Total loss in weight**	1.0 kgm.	1.8 kgm.	1.5 kgm.	1.6 kgm.

* Dog developed grave central nervous system and gastro-intestinal symptoms and refused to eat after 32 days.

** About 90 per cent of the loss in weight occurred during the last few days of life when there was anorexia, vomiting and diarrhea.

Two control dogs (dog 6 and dog 7) which received diet 324 showed definite prodromal symptoms after 16 and 17 days respectively, unmistakable mouth lesions after 36 days in each case and died after 56 and 38 days respectively. The general course of the decline was the same as that of the control dogs on diet 123 except that after the development of the lesions, the lesions progressed more slowly. Each of the control dogs lost weight during the test period.

A summary of the response of the four control dogs is given in table 1.

Dog 3, a 10-kgm. dog, was given diet 123 for 37 days at which time there had developed vermillion spots on the mucous membrane of the mouth which were accompanied by gastro-intestinal symptoms. Thereafter the dog was furnished with 300 mgm. per kilo of body weight per day of the vitamin concentrate in addition to daily portions of diet 123 corresponding to 80 calories per kilo of body weight. This dog was in buoyant spirits and in excellent physical condition at the end of the test,

174 days after the experiment was begun and had gained two kilos in weight during this time. The daily doses of vitamin concentrate were reduced to 200 mgm. per kilo per day after the first month of its administration without any detectable change in the condition of the dog. The dog's coat, like those of the other dogs receiving the vitamin concentrate, became remarkably thick and glossy and the eyes very bright and animated. The delay of 37 days before the administration of the vitamin concentrate accompanied by the signs and symptoms of black-tongue, as indicated, makes this experiment a more rigid test of the effectiveness of the vitamin concentrate than a strictly preventive test would have done. For all practical purposes it may be considered a successful curative test.

Dog 4, weighing 6.6 kilos, was given daily portions of diet 123 equivalent to 80 calories per kilo of body weight plus 300 mgm. of the vitamin concentrate per kilo of body weight from the beginning of the test. After one month diet 324 was substituted for diet 123. One month following this change the vitamin concentrate dosage was reduced to 200 mgm. per kilo of body weight per day. This dog was in excellent condition at the termination of the experiment at 138 days and had gained 0.7 kilo in body weight during this period.

Dog 5, weighing 7.1 kilos, was given daily portions of diet 324 in quantities corresponding to an 80 calorie intake per kilo of body weight for 20 days by which time vermillion spots had appeared on the mucous membrane of the mouth and gastro-intestinal symptoms had developed. Thereafter 300 mgm. of the vitamin concentrate per kilo of body weight were administered in addition to the basal ration, this quantity was subsequently reduced to a 200 mgm. level without apparent change in the condition of the dog. This dog was in excellent physical condition at the end of the test period, 110 days after the beginning of the experiment and had gained 0.9 kilo in body weight since the administration of the vitamin concentrate was begun.

In our opinion this concentrate is so clearly effective in creating the difference in the responses of the dogs on preventive or curative tests as compared with that of the control dogs as to make further extension in the duration of the experiments of no particular value.

A summary of the responses of the dogs receiving the concentrate of the heat-stable factors of the vitamin B-complex are given in table 2.

Twenty-eight day old white rats weighing 40 to 55 grams and of such nutritional background as we regularly employ for vitamin G researches were fed the black-tongue producing diets 123 and/or 324. These diets were of the same composition as those given to the dogs. Six rats of each sex were given diet 123 ad libitum and six of each sex received the same diet with 40 mgm. per day of the vitamin concentrate of the same lots

which were used for the tests of black-tongue prevention in dogs. Similarly, an equal number of each sex was given diet 324 alone and a further group diet 324 plus 40 mgm. per day of the vitamin concentrate. The

TABLE 2

Summary of dogs receiving black-tongue producing diets supplemented with vitamin G concentrate

	DOG 3	DOG 4	DOG 5
Diet no	123	123 and 324*	324
Quantity of vitamin G concentrate given per kgm. per day	300 mgm. 30th to 60th day, 200 mgm. thereafter	300 mgm. first to 60th day, 200 mgm. thereafter	300 mgm. 20th to 60th day, 200 mgm. thereafter
Duration of experiment	176 days	138 days	110 days
Total gain in weight	2.0 kgm.	0.7 kgm.	0.9 kgm.
General physical condition	Excellent	Excellent	Excellent

* Diet 123 was given for the first 30 days, diet 324 thereafter.

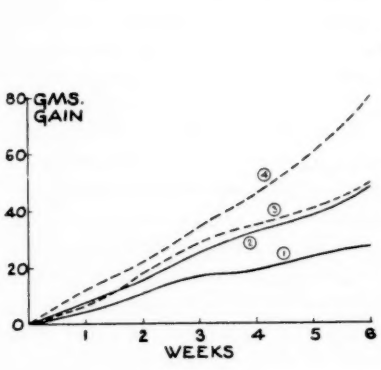

Fig. 1

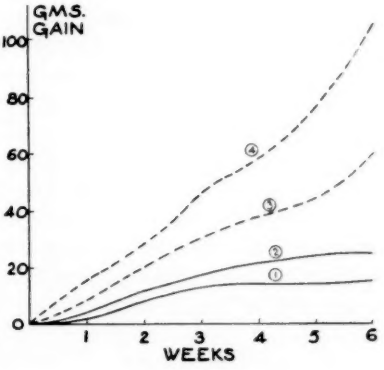

Fig. 2

Fig. 1. Curves *1* and *2* summarize the average growth rates of six young female and six young male rats, respectively, on black-tongue producing diet 123. Curves *3* and *4* are littermate control (female and male respectively) rats which received diet 123 plus 40 mgm. per day of the vitamin G concentrate.

Fig. 2. Curves *1* and *2* summarize the average growth rates of six young female and six young male rats, respectively, on black-tongue producing diet 324. Curves *3* and *4* are littermate controls of rats represented in curves *1* and *2* but which received in addition to diet 324, 40 mgm. per day of the vitamin G concentrate.

growth rates of these four groups of rats on the black-tongue producing diets 123 and 324 with and without administration of the vitamin concentrate are shown graphically in figures 1 and 2. The two sexes in each

case grew at sufficiently different rates to warrant separate plotting of their growth rates. The rats representing these four groups were littermate controls. The practice of coprophagy was eliminated in these tests. As is evident from these curves, the rats receiving diet 123 alone grew at a greater rate than those receiving diet 324 alone. However with the addition of equal amounts (40 mgm. per day) of the vitamin concentrate to these diets the rats on diet 123 grew less than those on diet 324. Neither of these diets is entirely devoid of the heat-stable factors of the vitamin B-complex as judged by these experiments since the rats receiving either diet 123 alone or diet 324 alone grew at slow rates. The growth rates, however, were much augmented by the addition of the vitamin concentrate.

We believe that the nutritional superiority of diet 324 in respects other than its vitamin G content as compared with diet 123, as shown in the experiments both with rats and with dogs, may be largely attributed to the higher protein content of diet 324. This nutritional difference between the two black-tongue producing diets supports the earlier indication of Goldberger and his associates that pellagra cases were benefited by the addition of protein to their diets even though the "P-P factor" was later found to be the specific dietary deficiency. This nutritional interrelation of protein and vitamin G has also been affirmed by Sherman and Derbigny (10) in experiments with rats.

Discussion. The most distinctive lesion of black-tongue is observed in the mucosa of the mouth. This lesion, however, occurs rather late in the progress of the disease and appears as reddened patches on the mucosa of the upper-lip, or of the cheek or floor of the mouth. With further progress of the disease there appear superficial necrotic areas within these reddened patches. It is probably because these lesions are so distinctive of the disease that the more disturbing prodromal symptoms involving the central nervous system and the gastro-intestinal tract have not been more strongly emphasized. Black-tongue should be regarded as a disease of severe systemic disturbances rather than a disease merely of severe buccal lesions. Epithelium and cells derived from epithelium embryologically are the elements of structure which suffer severely in black-tongue.

Our experiments, thus far, do not indicate whether only one or more than one of the heat-stable factors of the vitamin B-complex is involved in the prevention or cure of black-tongue. The parallelism in growth stimulating properties of the vitamin concentrate for rats and the black-tongue preventive or curative properties of this same concentrate for dogs would indicate that an identical substance (or substances) carried by this concentrate was the effective agent for promoting these respective responses in the two species of animals. We have also found that vitamin G (lactoflavin) alone, uncontaminated with other factors of the vitamin

B-complex, when given as a supplement to young rats on either one of the black-tongue producing diets, will augment growth.

The use of 200 mgm. of our vitamin concentrate per kilo of body weight of the dogs appears to allow for a considerable margin of safety since there was every indication that our treatment dogs were generously supplied with the black-tongue preventive factor.

The early manifestations in the control dogs which indicated a deficiency of the black-tongue preventive factor, whether or not the dogs had received the liver diet (conditioning diet) in a preliminary period, would indicate that the bodily stores of this factor were insufficient for the maintenance of health for longer than sixteen to nineteen days. The practical implications of this rapid depletion of bodily stores of black-tongue preventive factor should not be overlooked.

SUMMARY

A vitamin G concentrate obtained from low-lactose whey powder which carries, in addition to vitamin G (lactoflavin), at least one other heat-stable vitamin necessary for rat-growth, has been found effective for the prevention or cure of black-tongue. A daily quantity of 200 mgm. of this vitamin concentrate per kilo of body weight per day was more than sufficient to protect dogs against black-tongue.

The diets used for the production of black-tongue in dogs induced an inferior rate of growth when fed to young rats. These growth rates were augmented as a result of supplementing these diets with the vitamin concentrate which was preventive or curative for black-tongue.

There is evidence that the inclusion of liberal quantities of protein of good nutritive value decreases the vitamin G (and/or other heat-stable vitamins) requirements for the growth of young rats and slows up the progress of the development of the mouth lesions of experimental black-tongue.

We wish to express our thanks to the American Medical Association, Council of Pharmacy and Chemistry for a grant-in-aid of this research.

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CIRCULATORY AND VISUAL EFFECTS OF OXYGEN AT 3 ATMOSPHERES PRESSURE[1]

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In dogs anesthetized with sodium barbital, oxygen at a pressure of 4 atmospheres produces a fall in blood pressure and convulsive seizures which terminate in paralysis of respiration (Shaw, Behnke, and Messer, 1934). Since these phenomena are rapidly reversed when air replaces oxygen, the inference may be drawn that oxygen acts directly on the central nervous system, and possibly reflexly through the carotid sinuses.

In man oxygen at a pressure of 4 atmospheres induces either sudden syncope or convulsive seizures followed by complete recovery when air is again breathed (Behnke, Johnson, Poppen and Motley, 1935). In view of these striking phenomena, the question arose concerning the symptoms of oxygen toxicity for man at pressures less likely to produce sudden collapse.

The results reported in this paper indicate that oxygen at a pressure of 3 atmospheres brings about definite but rapidly reversible changes in man, namely, concentric contraction of the visual field, diminution of visual acuity, dilatation of the pupils, a rise in blood pressure, constriction of the facial vessels, and increased pulse rate.

EXPERIMENTAL METHOD. Four healthy young men breathed oxygen from either a closed or open system equipped with mask (4 experiments) or helment (5 experiments) for periods up to 4 hours at a pressure of 3 atmospheres (30 lb. gauge). To accomplish this they were placed in the large pressure chamber described by Thomson, Yaglou and Van Woert (1932). The observations include records of the leucocyte count, blood pressure, heart rate, respiratory rate and minute volume, acuity of vision, area of the visual field, and the appearance time of a negative after-image. The visual field of each eye was measured on a perimeter immediately before and after oxygen breathing. Visual acuity was tested by the ability to distinguish two black lines drawn parallel on white piece of cardboard and separated by a distance of 0.35 mm. The negative after-

[1] This research was aided by the Miriam Smith Rand Fund.

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image was induced by allowing the subject to fix on a red and green cross held at a distance of 1 meter from the eye for a period of 20 seconds.

RESULTS. The period of oxygen breathing can be divided into two intervals. The first covers 3 hours during which oxygen was well tolerated. The second comprises a period of impending collapse which comes on abruptly during the 4th hour. The detailed results of a representative experiment are given in table 1.

Oxygen breathing up to the 4th hour. The usual symptoms were moderate facial pallor and dilatation of the pupils, a rise in diastolic blood pressure of about 10 points, and impairment in visual acuity up to 25 per cent. In

TABLE 1

Circulatory and visual effects of oxygen at 3 atmospheres pressure (subject 1)

TIME	BLOOD PRESSURE	PULSE RATE	VISUAL ACUITY DECREASE	TIME OF NEGATIVE AFTER-IMAGE	REMARKS
a.m.			*per cent*	*seconds*	
10:49—air	132/86	96	0		10:50 to 2:15, subject felt well.
10:50—O_2					Facial pallor noted at 1:04
11:10	126/84	90			2:15, subject stated that his field of
11:45	115/90	75	3	8	vision was decreased and that his
p.m.					fingers and toes felt numb
12:15	110/86	75	10	16	2:19, feeling of dizziness and im-
12:47	114/88	63	20	18	pending collapse. Sense of pre-
1:15	124/84	57	28	18	cordial oppression, and inability
1:47	138/102	57	26	18	to cough. Numbness of fingers and toes. Intense facial pallor. Dilated pupils
2:00	120/94	63			2:23, field of vision contracted to
2:19	150/104	75	60	20	the 10° circle of perimeter chart
2:22		81			3:30, return of the visual field to
2:23	Off O_2				normal
2:35—air	140/92	81	40	7	

several experiments vision and blood pressure were not appreciably affected. There were usually no abnormal subjective symptoms during this period.

Oxygen breathing during the 4th hour. Progressive contraction of the visual field was a constant symptom during the 4th hour. Measurements on the perimeter immediately following removal of oxygen showed a concentric contraction for each eye ranging from one-half the initial area down to the 10° circle, as shown in figure 1. In a single experiment the contracted field suggested left temporal hemianopsia, and the right pupil was dilated to a greater degree than the left. Central vision for form and color was impaired but not seriously until the period of impending collapse,

when visual acuity was reduced as much as 60 per cent or even temporarily lost during the transfer from oxygen to air. A delay of 50 to 100 per cent in the time of appearance of the negative after-image paralleled the reduction of visual acuity. At the end of 2 out of 4 experiments the colors red and green were not recognized. An intense pallor of the face was present during the 4th hour, accompanied by wide dilatation of the pupils which reacted to light and accommodation. Both systolic and diastolic blood pressure readings were increased. In one experiment, however, in which

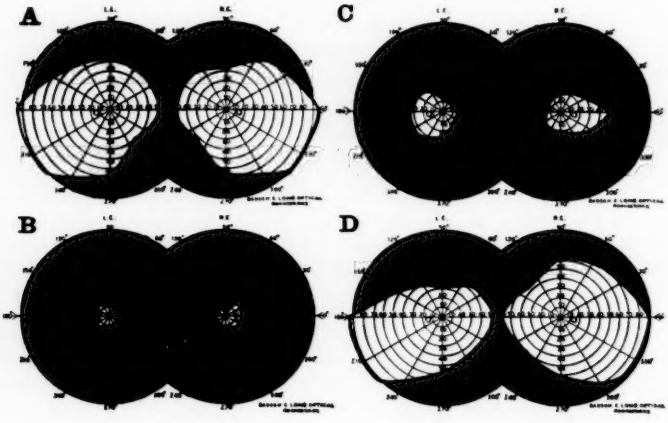

Fig. 1. Perimetric measurements made before and after 3½ hours' oxygen breathing at 3 atmospheres' pressure (30 lb. gauge). *A*, normal field limits; determinations made with the Ferree-Rand perimeter and exposure method with 7 foot-candles illumination. *B*, *C*, and *D*, field limits 5, 25, and 50 minutes, respectively, following 3½ hours' oxygen breathing; observations made at atmospheric pressure with a black perimeter of 25 cm. radius illuminated by a blue bulb placed behind and above the observer's head; moving stick stimuli were used and checked by the exposure method; test object was a white disc 6 mm. in diameter.

the blood pressure did not change the subject was in good condition at the end of 4 hours although contraction of the visual fields and facial pallor were present. Usually the abrupt onset of dizziness, nausea, and a feeling of impending collapse terminated the experiments in the fourth hour. Impending collapse was always signalized by an increase in pulse rate, rise of both systolic and diastolic pressure of 15 to 20 points, rapid contraction of the field of vision and failure in visual acuity for form and color. Although consciousness was retained at the end of all experiments the subjects looked dazed, and the delay in answering questions suggested partial stupefaction.

In two examinations retinal ischemia or constriction of vessels could not be detected.

The respiratory rate and minute volume were constant in all experiments. The leucocyte and differential counts did not show any unusual changes, and there were no subjective symptoms pointing to pulmonary injury.

In one experiment, after the subject breathed oxygen for 3 hours and 56 minutes without discomfort, the blower was stopped and the subject rebreathed the gas in the helmet. Within two minutes he approached a condition of collapse. It is believed that the increased carbon dioxide tension was responsible for the abrupt change.

The period of recovery. Gradual recovery took place within 20 to 60 minutes after air replaced oxygen. Nausea and dizziness disappeared within a few minutes but the return of blood pressure, pulse rate, size of the pupils, visual acuity, and facial color to normal took place concurrently over a considerably longer period of time. The contracted visual fields usually regained their initial limits within an hour (fig. 1). The period of recovery was roughly proportional to the time between the onset of visual field contraction and the termination of the experiment. The significant point about recovery was the feeling of alertness and stimulation.

Calibre of the pial vessels of the cat in relation to oxygen at a pressure of 4 atmospheres and to increased carbon dioxide tension. In order to determine the effect of oxygen and of oxygen and carbon dioxide on blood vessels the pial arteries of the cat were observed through a window placed in the skull according to the method of Forbes (1928). The observations indicated that oxygen breathing at a pressure of 4 atmospheres did not appreciably alter the calibre of the pial arteries. The action of carbon dioxide (approximately 60 mm. tension) in combination with oxygen resulted in a dilatation of the arteries followed by constriction when carbon dioxide was removed (fig. 2). While the experiments are too few for conclusions to be drawn with respect to the action of oxygen at high pressure on pial vessels, the dilating effect of carbon dioxide in combination with a high pressure of oxygen is definite and in accord with the results of other investigators working with normal oxygen tensions.

Discussion. Severe functional disturbances similar to those associated with high oxygen pressure are without parallel in pharmocologic reactions in regard to the complete and rapid recovery which invariably follows. Temporary oxygen deprivation or withdrawal of cerebral blood supply for very short periods of time perhaps most closely simulate the oxygen effects.

Oxygen toxicity in relation to circulatory changes. Tolerance for oxygen at a pressure of 3 atmospheres is closely related to the stability of blood pressure and pulse rate. In the experiments of 4 hours' duration, which were symptomless except for contraction of the visual field, the blood pressure

remained constant. The circulatory disturbance in man associated with the toxic action of oxygen is essentially a peripheral vasoconstriction. At a pressure of 3 atmospheres the period of impending collapse was always signalized by the abrupt rise of both diastolic and systolic blood pressure, increased pulse rate, facial pallor, and dilatation of the pupils—symptoms which suggest stimulation of the sympathetic nervous system. At a pressure of 4 atmospheres, a rise in blood pressure (not previously reported) from 116/86 to 130/104 immediately preceded a violent convulsive seizure (Behnke et al., 1935).

Whether the circulatory changes are direct effects of the high oxygen pressure or compensatory reactions remains to be determined. The experimental results, however, bring up the fundamental question whether

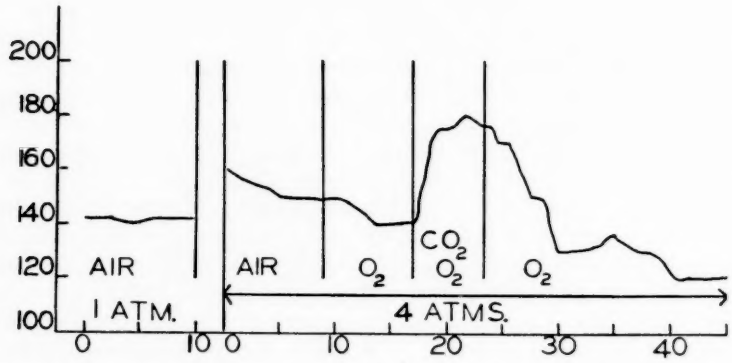

Fig. 2. Changes in the diameter of a pial arteriole of a cat breathing a 2 per cent carbon dioxide (equivalent to 8 per cent carbon dioxide at 1 atmosphere) and 98 per cent oxygen mixture at 4 atmospheres' pressure. *Ordinate*, diameter in microns; *abscissa*, time in minutes.

oxygen acts directly on nervous tissue to produce at 4 atmospheres the convulsive seizure and at 3 atmospheres contraction of the visual field, or whether these phenomena are the result of cerebral and retinal angiospasm induced by oxygen. Aid in answering this question is afforded by a consideration of the effect of carbon dioxide on cerebral vessels in relation to oxygen toxicity. The constriction of blood vessels would not, of course, deprive the brain of oxygen in view of the high partial pressure of this gas in the arterial blood (23 times the normal tension), but would tend to limit the supply of other necessary substances and hinder the removal of metabolites.

Action of carbon dioxide on cerebral vessels. The experiments of Gibbs, Gibbs, and Lennox (1935) indicate that human cerebral blood flow is

increased by raising the alveolar carbon dioxide tension. In cats, increased carbon dioxide tension dilates the pial vessels (Forbes, 1928; Wolff and Lennox, 1930) and increases the blood flow in the medulla and hypothalamus (Schmidt and Pierson, 1934; Schmidt, 1934–1935). Conversely, decreased carbon dioxide tension constricts pial vessels and decreases blood flow.

Carbon dioxide in relation to high oxygen pressure. From the observations reported in this paper on the pial vessels of the cat, the dilating action of carbon dioxide on arterioles is not altered by high oxygen pressure. The effect of carbon dioxide on cerebral vessels and blood flow offers, therefore, a partial explanation of the finding of Shaw et al. (1934) that with an oxygen pressure of 4 atmospheres, convulsive seizures and a fall in blood pressure can be rapidly induced in anesthetized dogs by raising the alveolar carbon dioxide tension to 65 mm., or prevented by lowering the carbon dioxide tension to 22 mm. From this fact it is inferred that carbon dioxide renders a given oxygen tension more toxic by increasing cerebral blood flow, and that decreasing cerebral blood flow by lowering the alveolar carbon dioxide tension will render the given oxygen tension less toxic.

That carbon dioxide increases oxygen toxicity in man is inferred from the condition of impending collapse which was rapidly brought about by stopping the circulation of oxygen during the 4th hour and allowing rebreathing to take place for a period of 2 minutes. If carbon dioxide dilates retinal and cerebral vessels in man and increases blood flow when oxygen is breathed at a pressure of 3 and of 4 atmospheres, then it can be concluded that cerebral and retinal angiospasm are not responsible under these circumstances for the toxic action of oxygen. The inference follows that oxygen acts directly on nervous tissues, and that peripheral vasoconstriction is probably a compensatory reaction.

In a study of the physicochemical reactions brought about in the nerve cell by high oxygen pressure, certain essential facts should be kept in mind: *a*, the action of oxygen is characterized by severe functional disturbances without apparent structural injury to nervous tissue; *b*, a latent period of about 3 hours at a pressure of 3 atmospheres and of 45 minutes at a pressure of 4 atmospheres precedes severe toxic symptoms in man; *c*, the circulatory, visual, and convulsive symptoms which lead to the collapse of the individual appear rather abruptly during the course of oxygen breathing; *d*, complete recovery is promoted by the substitution of air for oxygen, and requires a period of time roughly proportional to the duration of the toxic symptoms, i.e., 10 to 60 minutes; *e*, in man the sympathetic division of the autonomic nervous system is apparently stimulated since a rise in blood pressure, increase in pulse rate, and dilatation of the pupils accompany visual loss and mental impairment at a pressure of 3 atmospheres, while at a pressure of 4 atmospheres a rise in blood pressure pre-

cede a convulsive seizure; recovery, moreover, is attended by a feeling of stimulation; *f*, in dogs anesthetized with sodium barbital, a fall in femoral or carotid blood pressure is the constant and early sign of oxygen toxicity and always precedes the convulsive seizure; *g*, increased carbon dioxide tension hastens the fall in blood pressure and the convulsive seizure, while decreased carbon dioxide tension delays or prevents these symptoms.

SUMMARY

Oxygen at a pressure of 3 atmospheres (30 lb. gauge) can be breathed by healthy men for 3 hours without distressing symptoms. During the 4th hour a progressive contraction of the visual field with dilatation of the pupils and some impairment in central vision is the most constant criterion of oxygen toxicity.

Circulatory changes indicative of peripheral vascular constriction are associated with the visual impairment, and culminate during the 4th hour in an abrupt rise of systolic and diastolic blood pressure, increase in pulse rate, and extreme pallor of the face. At this stage the subjects experience dizziness and a feeling of impending collapse. A condition of partial stupefaction is indicated by the facial expression and the slowed mental responses.

Rapid and complete recovery attended by a feeling of alertness and stimulation takes place within an hour after air is substituted for oxygen.

We wish to express our appreciation and thanks to Dr. Marion R. Stoll of the Massachusetts Eye and Ear Infirmary for the perimetric studies, figure 1.

We wish to acknowledge our indebtedness to The Linde Air Products Company who supplied the oxygen used in these experiments, and to D. E. Billington, W. T. Denyer, B. L. Fahey, and F. Murray of the United States Navy for their coöperation throughout the experimental work.

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ON THE QUESTION OF DUPLICATION OF SYMPATHETIC INNERVATION TO BILATERALLY INNERVATED STRUCTURES

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In a previous report (Dye, 1935) it has been shown that the remote and local sympathin effects obtainable from unilateral stimulation of a given anatomical structure (or group of structures) through its sympathetic nerves may not be affected by previous exhaustion of the same structure due to stimulation of the corresponding nerves of the opposite side. This would indicate that there is no duplication of sympathetic innervation in bilaterally innervated structures. The present investigations were undertaken to study this problem further. If neither preganglionic nor postganglionic duplication of sympathetic innervation exists, the sympathin effects obtainable from simultaneous bilateral stimulation of a given sympathin source should be the summated effects of the two unilateral effects taken singly. Duplication of innervation would lead presumably to less than the expected summation effects.

METHODS. Cats, under dial anesthesia (Ciba), were used. As indicators of sympathin the following were employed: the nictitating membrane (n.m.) which had been sensitized by previous removal (4 to 11 days) of the superior cervical ganglion, blood pressure (b.p.) and heart acceleration (h.a.). Cocaine was usually employed to further sensitize the preparation (Rosenblueth and Cannon, 1932). The sources of sympathin to be summated were the effectors innervated by the two splanchnic nerves (spl.), by the internal branches of the stellate ganglia, primarily cardioaccelerator nerves (c.a.) and by the two lower abdominal sympathetic nerves (las.). All nerves were crushed or cut centrally prior to stimulation. The r. and l. nerves in each case were stimulated separately and simultaneously, the order of stimulation being varied. Supermaximal stimuli were always used. In order that the stimulating shocks may be applied to the two contralateral nerves at exactly the same instant, a double inductorium (after Vintschgan) was employed. The primary current was 6 volts, the secondary coils were placed at 12 cm. unless other-

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wise indicated (approximately equivalent to 8 cm. on the Harvard inductorium). The stimulation frequency was maintained at 20 per second, the quantity of sympathin produced was varied by altering the duration of the stimulation. Shielded electrodes were always employed.

Isotonic responses of the n.m. were recorded on a kymograph, the magnification being 20 times. B.p. was similarly recorded by a Hürthle type manometer which was calibrated at each experiment against a mercurial manometer. The h.a. was counted from the b.p. records. The measurements are expressed as maximal height of the n.m. records in mm., maximal rise of b.p. in mm. Hg and h.a. in beats per minute.

RESULTS. 1. *A comparison of the n.m. responses following stimulation of the r. and l. spl. nerves separately and simultaneously.* From experiments 1 and 2, table 1, it will be seen that the output of sympathin, as indicated by the n.m. and b.p. responses, is usually approximately the same whether the r. or l. spl. is stimulated. With longer stimulations, the quantities of sympathin produced being relatively large, the observed summations (B) from simultaneous stimulation of the two spl. were invariably less than the theoretical linear summation (A) of the two separate n.m. responses. As the total quantity of sympathin produced was diminished, the observed summations approximated or exceeded unity. In all cases where just subthreshold stimulation of one and minimal stimulation of the other spl. for n.m. excitation occurred separately, simultaneous stimulation of the two nerves at the same frequency, duration and strength of stimulus (same quantity of sympathin) gave summated responses which were greater than linear. In experiment 1 the effective threshold stimulation increased progressively as the experiment progressed. Unilateral stimulations which were just subthreshold for the n.m. gave positive n.m. responses when summated.

2. *Local summation effects of sympathin as indicated by b.p. following spl. stimulation.* An analysis of the vaso-constrictor responses (local sympathin effects) following stimulation of the spl. nerves, as indicated by b.p., led to the conclusion that all stimulations which were above the threshold for remote sympathin effects (n.m.) invariably gave local b.p. summation responses which were less than linear. In order that a complete study of the local effects of sympathin may be made, it was necessary to obtain b.p. responses varying from minimal to maximal. This was done by varying the stimulation time, the frequency remaining constant. The data from six typical experiments are given in table 1. The following results were observed:

1. With both spl. nerves crushed, a general deterioration of the preparation usually occurred. There was not only a gradual decline in the resting b.p. level, but a gradual diminishing effect of a given stimulus on the elevation of b.p.

TABLE 1

Summation effects of sympathin from the splanchnic area

EXPERIMENT NUMBER	MID-PERIOD TIME FROM START OF EXPERIMENT, HOURS: MINUTES	STIMULATION TIME, SECONDS, 20 PER SECOND	INDICATOR	R. SPL.	L. SPL.	A SUM OF R. + L. SPL.	B ACTUAL SUMMATION R. + L. SPL.	RATIO $\frac{B}{A}$
				mm.	*mm.*	*mm.*	*mm.*	
1	0:17	30	b.p.	73	70	143	85	0.59
			n.m.	34	28	62	35	0.56
	0:47	30	b.p.	66	55	121	85	0.70
			n.m.	18	0	18	29	1.61
	1:04	60	b.p.	102	92	194	115	0.59
			n.m.	22	0	22	30	1.37
	1:38	60	b.p.	65	73	138	90	0.65
			n.m.	0	0	0	19	
	2:30	90	b.p.	75	103	178	130	0.73
			n.m.	0	0	0	30	
2	0:22	30	b.p.	95	100	195	140	0.72
			n.m.	55	54	109	72	0.66
	1:06	15	b.p.	80	70	150	100	0.67
			n.m.	41	41	82	64	0.78
	2:00	15	b.p.	80	75	155	100	0.65
			n.m.	38	37	75	45	0.60
	2:40	10	b.p.	45	57	102	80	0.78
			n.m.	0	7	7	34	4.86
3	0:11	30	b.p.	110	107	217	130	0.60
	0:29	20	b.p.	85	65	150	110	0.73
	1:11	30	b.p.	40	30	70	70	1.00
	1:48	20	b.p.	47	25	72	68	0.94
	2:30	10	b.p.	35	25	60	58	0.97
4	0:10	30	b.p.	83	85	168	123	0.73
	0:24	1	b.p.	7	9	16	16	1.00
	0:32	0.5	b.p.	3.5	4	7.5	7.5	1.00
5	0:18	30	b.p.	90	85	175	97	0.55
	0:46	5	b.p.	40	15	55	53	0.96
	1:02	15	b.p.	46	47	93	85	0.91
	1:22	20	b.p.	80	36	116	90	0.78
6	0:12	30	b.p.	100	50	150	85	0.57
	1:45	15	b.p.	50	35	85	65	0.76
	1:58	5	b.p.	27	10	37	28	0.76
	2:10	5	b.p.	23	6	29	25	0.86
	2:30	2	b.p.	20	8	28	26	0.93
	2:40	2	b.p.	20	4	24	25	1.04
	2:45	1	b.p.	9	0	9	9	1.00

No cocaine in 3 and 6; Hns. cut in 1 and 3.

2. Longer stimulation periods, greater quantities of sympathin, invariably gave less than linear summation effects.

3. Intermediate stimulations or intermediate quantities of sympathin usually gave summated effects which were approximately linear.

4. Minimal separate stimulations with minimal amounts of sympathin similarly gave summated effects which were approximately linear. This fact is of primary importance.

5. With minimal stimulation of one and just subminimal stimulation of the other spl. the summated effects were again linear and not more than linear as with the remote sympathin effects (n.m.).

6. No b.p. response occurred from simultaneous stimulation of both spl. nerves with just subminimal stimuli.

7. Unlike the remote effects of sympathin, the local effects never lead to more than linear summations following brief stimulations.

TABLE 2

Summation effects of sympathin from the las.

EXPERIMENT NUMBER	MID-PERIOD TIME FROM START OF EXPERIMENT, HOURS: MINUTES	STIMULATION TIME, SECONDS, 20 PER SECOND	INDICATOR	R. LAS.	L. LAS	A SUM OF R. + L. LAS.	B ACTUAL SUMMATION R + L. LAS	RATIO $\frac{B}{A}$
				mm.	*mm.*	*mm.*	*mm.*	
7		120	b.p.	30	25	55	56	1.02
			n.m.	5	0	5	19	3.80
8	0:11	60	b.p.	35	42	77	55	0.71
	0:42	60	b.p.	19	19	38	34	0.89
	1:02	10	b.p.	15	17	32	25	0.78
	1:58	2	b.p.	10	7	17	17	1.00
	2:12	1	b.p.	9	4	13	12	0.93

Secondary coil at 10 cm. (equivalent to about 6.5 to 7 cm. on the Harvard inductorium).

3. *Summation effects of sympathin from the las.* From the data given in table 2, it will be seen that essentially the same summation effects were obtained from stimulation of the las. as from stimulation of the spl. Outputs of sympathin large enough to induce linear or less than linear summation responses from the n.m. were not obtained. In experiment 7 two minutes' stimulation gave linear b.p. summation effects and more than linear n.m. responses. In experiment 8, when the stimulations were inadequate to elicit remote (n.m.) sympathin responses, the local vasoconstrictor effects (b.p.) were similar to those obtained for the splanchnic area under similar conditions.

4. *Summation effects of sympathin from the c.a. on the n.m., b.p., and h.a.* A study of the summation effects from the c.a. was desirable since these nerves are composed presumably of postganglionic fibers. With the

secondary coil at 11 cm. (expt. 9, table 3), both the b.p. and n.m. responses were approximately linearly summated. Increasing the strength of the stimulus to 10 cm. resulted in b.p. effects whose summations were less than linear. The corresponding n.m. responses (remote effects of sympathin)

TABLE 3

Summation effects of sympathin from the cardio-accelerator nerves

EXPERIMENT NUMBER	MID-PERIOD TIME FROM START OF EXPERIMENT, HOURS: MINUTES	STIMULATION TIME, SECONDS, 20 PER SECOND	SECONDARY COIL DISTANCE	INDICATOR	R. C. A.	L. C. A.	A SUM OF R. + L. C. A.	B ACTUAL SUMMATION R. + L. C. A.	RATIO $\frac{B}{A}$
			cm.		*mm.*	*mm.*	*mm.*	*mm.*	
9	0:10	60	11	b.p.	28	22	50	49	0.98
				n.m.	12	6.5	18.5	19	1.03
	0:30	60	10	b.p.	33	47	80	55	0.69
				n.m.	8	19	27	24	0.89
	0:52	30	10	b.p.	23	20	43	28	0.65
				n.m.	3	3.5	6.5	7	1.08
	1:12	60	10	b.p.	17	18	35	30	0.86
				n.m.	3	5	8	10	1.25
10		8	11	h.a.	48	30	78	57	0.73
		12	11	h.a.	54	30	84	60	0.71
		4	11	h.a.	44	6	50	48	0.96
		2	11	h.a.	23	9	32	32	1.00
		10	11	h.a.	51	12	63	53	0.84
		0.5	11	h.a.	6	0	6	6	1.00
		2	10	h.a.	36	6	42	39	0.93
		20	11	h.a.	60	24	84	54	0.64
11		10	10	h.a.	10	26	36	30	0.83
		4	10	h.a.	9	7	16	15	0.94
		4	10	h.a.	9	6	15	16	1.07
		2	10	h.a.	4	2	6	6	1.00
		2	10	h.a.	3	6	9	9	1.00*
		4	10	h.a.	6	14	20	21	1.05
		6	10	h.a.	8	17	25	18	0.72
		8	10	h.a.	9	24	33	26	0.79
		10	10	h.a.	10	24	34	27	0.79
		16	10	h.a.	10	28	38	30	0.79
		20	10	h.a.	12	34	46	34	0.74
		4	10	h.a.	3	7	10	10	1.00

* Adjusted the electrodes.

No cocaine was used in 10 and 11. Listed in the order taken.

were at first less than linear, but regained and finally exceeded linear proportions as the preparation became less sensitive.

B.p. responses following stimulation of the c.a. are due to the combined action of two factors; first, increased heart output accompanying cardio-

acceleration (local action of sympathin); and second, the remote effects of sympathin throughout the body on the vessels which receive a sympathetic vaso-constrictor supply. The remote effects probably predominate since the effects outlast considerably the stimulation time. Furthermore, the latent period and the duration of the response parallel those of the n.m.

When the h.a. is plotted against the stimulation time (quantity of sympathin) the curve is a rectangular hyperbola. The accelerator effects of the r. and l. c.a. are quite unequal, however. Stimulation of the r. c.a. produces considerably more cardio-acceleration than stimulation of the l. c.a. It is generally recognized that the r. c.a. supplies many fibers while the l. c.a. supplies relatively few fibers to the sino-auricular node in most animals. Due to this anatomical difference, the l. c.a. had relatively little influence on the summation effects when both c.a. were stimulated simultaneously (expt. 10). With brief stimulations the accelerator influence of the l. c.a. failed entirely or was slight while that for the r. c.a. remained relatively high. The significance of these facts is obvious when it is remembered that the curve illustrating the relationship between the quantity of sympathin produced and the response elicited is a rectangular hyperbola (Rosenblueth, 1932). To overcome this difference between the action of the nerves from the two sides, a single branch of the r. c.a. was stimulated (expt. 11). Under these conditions the separate effects of unilateral stimulation were more nearly equal. The summated effects were again less than linear for longer stimulations, larger quantities of sympathin, and linear for brief stimulations. More than linear relations were never encountered.

Since the local effects of sympathin, as indicated by the stimulation of postganglionic fibers (c.a.), gave linear and not less than linear summations with brief stimulations, it seems logical to conclude that duplication of postganglionic innervation to the heart from contralateral c.a. nerves does not exist. Furthermore, the summation effects obtained from these nerves (postganglionic fibers) are indistinguishable from those obtained from the spl. and las. (preganglionic fibers) under similar conditions.

Discussion. Two possibilities of duplication of sympathetic nerve supply to an organ may exist; first, from postganglionic fibers; and second, from synaptic connections between a given ganglion cell, or group of cells, and a preganglionic fiber from the r. and l. sides. The data given in section 4 indicate clearly the absence of such postganglionic duplications. Cannon (1933) has recently reviewed the evidence which not only indicates that there is no duplication of sympathetic innervation postganglionically, but that not all of the smooth muscle cells have a direct innervation.

Using the sensitized n.m. as an indicator, Rosenblueth and Morison (1934) found the summated effects of sympathin when obtained from two different anatomical sources (r. c.a. and r. spl.) to be greater than linear

for small, approximately linear for intermediate and less than linear for larger separate effects. They ascribe these differences to threshold and concentration factors. Cocaine reduces the threshold level (Rosenblueth and Cannon, 1932; Rosenblueth and Morison, 1934). The data for remote sympathin summation effects reported in sections 1, 3 and 4 of the present paper show similar summation phenomena. It seems evident that the summations obtained from simultaneous stimulation of the r. and l. sympathetic nerve supply to a single bilaterally innervated organ are identical with those obtained from simultaneously stimulating the sympathetic innervations of two separate organs. This would indicate that each set of nerves innervates separate and specific effectors in such an organ and that there is no bilateral duplication of innervation.

Since all postganglionic nerve impulses would be maximal following preganglionic stimulation, the quantity of sympathin produced and the effective response would be proportional to the number of postganglionic fibers involved and the frequency of the impulses. From their study of the functional relations between the preganglionic and postganglionic fibers in the superior cervical ganglion, Bishop and Heinbecker (1932) concluded that each preganglionic impulse is followed by a single postganglionic discharge. There is no spread from one pathway to another and there is no after-discharge. One preganglionic fiber connects with many ganglion cells and activates them all at each discharge. Even though a preganglionic nerve impulse from each side is sent to a given group of ganglion cells, it is not likely that more than one impulse would be set up in each postganglionic nerve fiber concerned so long as they arrived at the ganglion simultaneously or within the refractory period of the ganglion. This condition was presumably obtained in the present experiments by the use of a double inductorium which made it possible to stimulate the two contralateral nerves by the same current at exactly the same instant. Bilateral duplication of preganglionic fiber connections with ganglion cells would lead to relatively large unilateral stimulation responses and less than linear summation effects with all durations of stimulation. This condition did not prevail with brief stimulations.

Increasing quantities of sympathin also lead to less than linear summation effects (Rosenblueth and Morison, 1934). This holds true for both local and remote sympathin responses. This concentration factor, however, may be essentially eliminated by reducing the stimulation and hence the sympathin production so as to correspond to the steep ascent of the hyperbolic curve which expresses the relationship between the quantity of sympathin produced and the response (Rosenblueth, 1932). This obtains for minimal local effects and for intermediate remote effects, but not for minimal remote effects since these give more than linear summation due to threshold factors (Rosenblueth and Morison, 1934).

Remote sympathin summation effects are temporal summations. The quantity of sympathin diffusing into the blood, whether from one or more sources, determines the magnitude of the response. The local responses to sympathetic nerve stimulations are as truly sympathin effects as are the remote responses and the threshold factors are relatively negligible since here they are concerned primarily with local spread and irritability of the effectors. If then there is neither preganglionic nor postganglionic duplication of sympathetic innervation, as the evidence strongly indicates, the effectors innervated by the contralateral nerves must be entirely separate. Furthermore, if the stimulations of these nerves were such as to produce minimal quantities of sympathin locally, where it may be reasonably assumed that it will be used in effecting a stimulation or that it will be destroyed locally (Rosenblueth, 1932; MH = M + H), the response so elicited should approximate, if not be, true local effects with no spread of chemical stimulation between adjacent nerve terminals and their surrounding muscle fibers. Under these conditions summation responses would be due solely to the sum of spatial effects, spatial summation, and should approximate linear proportions. Stronger stimulations with consequent larger production of sympathin would not only lead to local spatial summations, but to local diffusion and concentration factors together with remote effects. These conditions lead to temporal and less than linear summation.

The observation was soon made that under no conditions were the local summation effects more than linear. Longer stimulations led to less than linear summation responses due to concentration factors, brief stimulations led to linear summations. These conditions meet the requirements for a separate and distinct distribution of sympathetic nerve supply from the two sides, but are incompatible with contralateral duplication of sympathetic innervation.

SUMMARY

The problem of duplication of sympathetic innervation of bilaterally innervated anatomical structures has been attacked experimentally from a study of the local and remote effects of sympathin following unilateral and bilateral stimulations. The n.m., b.p. and h.a. responses in sensitized cats were employed as indicators of the quantity of sympathin produced. The effectors innervated by the two spl. nerves, by the internal branches of the stellate ganglia (primarily c.a.) and by the two las. were employed as sources of sympathin.

Unilateral stimulations which were adequate to give large remote effects (n.m.) always gave less than linear summations, those which produced intermediate responses gave summated effects which approximated linear proportions, while those which gave minimal responses or which were just

below the threshold for the n.m. gave more than linear effects when summated. The local effects of sympathin on b.p. and h.a. were similar to the remote ones with one fundamental exception, namely, that with the former there was never more than linear summation with minimal or just subthreshold stimulations. These are always approximately linear.

The problem of preganglionic and postganglionic duplication of sympathetic innervation of smooth muscle is discussed briefly. On the assumption that brief stimulations lead to restricted local sympathin effects, hence are capable of only spatial summation, it is concluded that duplication of sympathetic innervation does not exist.

This work was done at the suggestion of Dr. W. B. Cannon. I wish to express appreciation to him and to Dr. A. Rosenblueth for their many helpful suggestions and criticisms.

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DAILY ERYTHROCYTE COUNTS IN MENSTRUAL AND INTERMENSTRUAL PERIODS[1]

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In her study of transfused blood, Ashby (1921) concluded that 1, "Group IV transfused blood in a recipient of unlike group is eliminated by a blood-destroying activity of the body," and that 2, "This blood-destroying activity is periodic both in men and women, and in women coincident with menstruation." This study was undertaken to see 1, whether the number of erythrocytes would show any rhythm when daily counts were made over fairly long periods of time; 2, whether it was possible to correlate changes in the volume, the hemoglobin content and the number of reticulocytes with the variations in the number of red blood corpuscles, and 3, whether the menstrual period influenced the daily curve. The results showed that 1, the curves plotted for the numbers of erythrocytes possessed waves of varying lengths with small fluctuations from day to day; 2, no correlation between the numbers of corpuscles and the total volume of cells, the hemoglobin content and the numbers of reticulocytes could be demonstrated with the methods employed, and 3, the part of the curve during the menstrual period of normal women was not different from that during the intermenstrual portion of the cycle but was itself a part of the whole. This latter interpretation explains the conflicting data on the relation of menstruation to the numbers of erythrocytes. Increases and decreases during the period can only be considered as parts of the normal curve.

METHOD. The total erythrocyte counts were made according to the method of Smith (1931). The hemoglobin content was estimated by use of the Dubosc colorimeter with the Bausch and Lomb hemoglobin attachment. The Van Allen (1924–1925) capillary hematocrit tube was used for the total cell volumes. The reticulocytes were counted in wet smears according to the method of Friedlander and Wiedemer (1929), the per-

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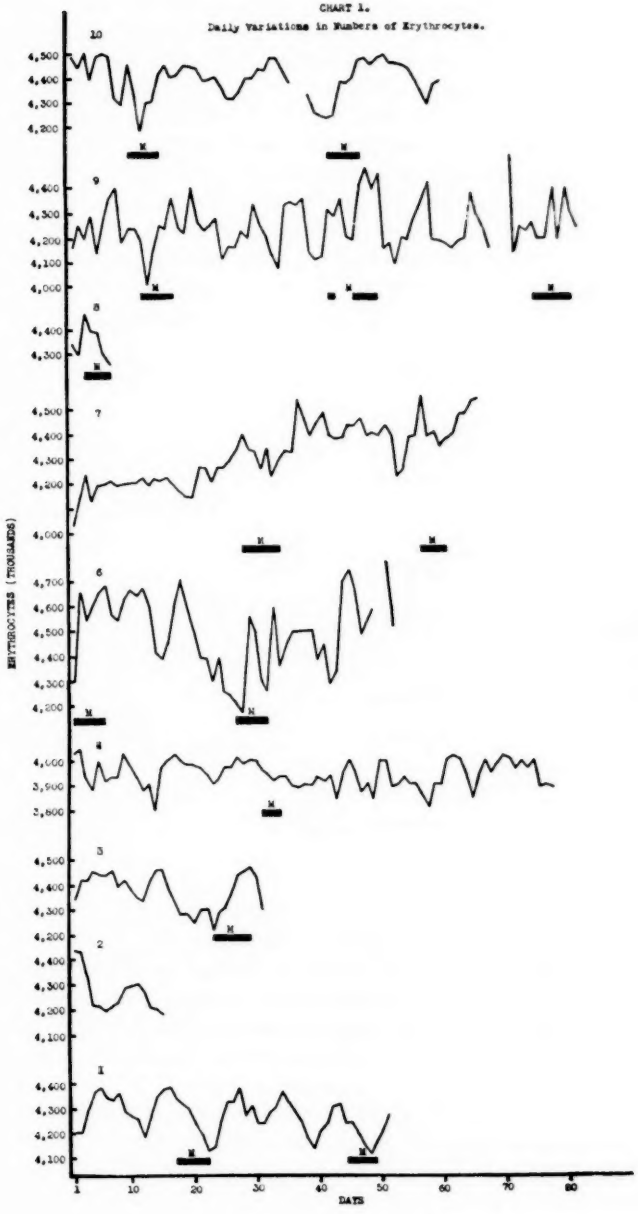
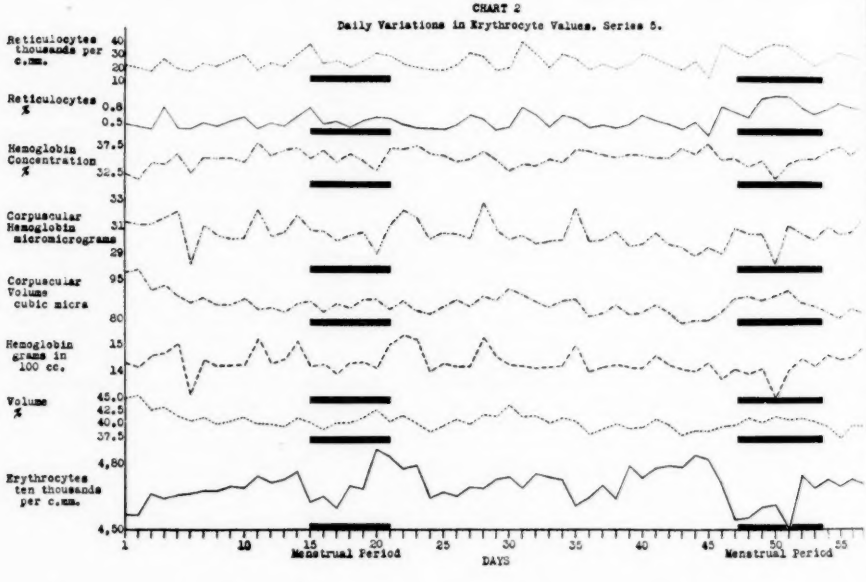
CHART 1.

Daily Variations in Numbers of Erythrocytes.



centage being determined from the number in 1000 red cells. The corpuscular values were derived by use of the formulae of Wintrobe (1929).

RESULTS AND DISCUSSION. The ten curves for the total counts of erythrocytes (charts 1 and 2) showed that the daily variations were parts of larger fluctuations of several days' duration. Although the shape of the curves was irregular and not characteristic for any one person, the form was not that of a saw-tooth edge due to sharp differences every 24 hours but a pattern made up of larger waves extending over a few to many days. The zone within which these fluctuations occurred even over fairly long periods of time was relatively narrow. The greatest range recorded in



these studies was 600,000 cells (chart 1, series 6 and table 1) and the least for a long series, 220,000 cells (chart 1, series 4 and table 1). These figures are not very different from those found for 8-hour counts during the day. According to Smith (1931) the average daily range in 19 series was 251,000 $\pm$18,000 cells with a maximum range of 490,000. The abrupt falls in the curves may be due to periodic blood destruction but there are no data to support this assumption.

The curves for the volume of cells, the hemoglobin content and the numbers of reticulocytes had no individual characteristics, nor any correlation with the fluctuations in the numbers of erythrocytes. Chart 2 is given as a representative example of a series (series 5) in which all determinations

were made. In this series the average number of erythrocytes was 4,693,000 with a coefficient of variation of 1.68 per cent; the average total volume of cells, 40.45 per cent, c.v. 3.70 per cent; the average hemoglobin content, 14.30 grams, c.v. 3.49 per cent; the average number of reticulocytes, 27,400, c.v. 33.45 per cent. In series 7 where there was a gradual rise in the total number of erythrocytes paralleling an increase in weight, there was also a corresponding decrease in the number and percentage of reticulocytes. A summary of the data for the other series is given in table 1.

The parts of the erythrocyte curves during menstruation had no constant characteristics in themselves nor any differences from other portions.

TABLE 1

Summary of results

SERIES	SUBJECTS	AGE	DATE OF FIRST DETERMINATION	DURATION OF STUDY	NO. OF MENSTRUAL PERIODS	RANGE RED CELLS 10,000's	RANGE VOLUME	RANGE Hb	RANGE RETICULOCYTES	TIME OF EXAMINATION
				days			*per cent*	*grams*	*per cent*	
1	A	37	1/ 8/30	51	2	4,12–4,39		13.10–13.95	0.5–1.3	8 a.m.
2	A		4/ 1/30	15		4,18–4,44				8 a.m.
3	A		2/ 2/31	30	1	4,22–4,61	38.2–44.6	13.70–15.25	0.73–1.38	8 a.m.
4	B	22	4/24/30	78	1	3,81–4,03	38.4–44.5	11.18–13.96		9 a.m.
5	C	22	1/13/31	57	2	4,49–4,87	37.7–45.2	13.13–15.39	0.28–1.05	8 a.m.
6	D	21	1/29/30	52	2	4,17–4,77			0.55–1.25	
7	D		9/27/30	66	2	4,03–4,58		11.86–14.91	0.6 –2.55	8 a.m.
8	E	20	8/ 9/29	7	1	4,26–4,46		13.63–13.88		*
9	E		9/21/29	80	3	4,08–4,52		13.22–13.88	0.4–3.1	†
10	F	21	10/22/29	58	2	4,18–4,51		13.10–13.79		8 a.m.

* Four counts each day, 9, 10, 11, and 12 a.m.

† Most at 5 p.m., some during a.m.

In the sixteen menstrual periods studied, some showed increasing and some decreasing numbers of cells, and others, irregular fluctuations. The amount of hemorrhage in normal persons seemed not important as that factor varied from a slight amount in some to a rather large loss in one. One person (chart 1, series 1, 3, and 8, 9) did not always have the same pattern in the menstrual part of the curve. Neither did the other values studied exhibit any differences due to menstruation. If as Ashby (1921) thought the blood destroying activity of the body was coincident with menstruation in women, some change might be expected in the number of reticulocytes. This was not found to be true.

Previous studies on the influence of menstruation on the numbers of

erythrocytes have been limited mostly to premenstrual and menstrual changes. There have been recorded premenstrual and menstrual rises and falls in the number of cells with the majority finding a decrease most usual during menstruation (Detre, 1928; Dupérié, 1878; Holler, Melicher and Reiter, 1924; Krutchenkoff, 1909–1910; Michels, 1931; Piney, 1931; Pölzl, 1910; Reinert, 1891). Very little work has been done on the hemoglobin content, cell volume, the corpuscular values and the number of reticulocytes during menstruation. Gumprich (1913–1915) and Reich and Green (1932) found no relation between the number of red blood corpuscles and the hemoglobin and the menstrual process. Pölzl (1910) stated that the changes in hemoglobin did not parallel the fluctuations in the erythrocytes while Detre (1928) believed both the hemoglobin and the volume fell along with the numbers of cells. Dupérié (1878) observed that there was an increase in the small newly-made cells during the period. Reich and Green (1932) in their biweekly determinations made counts of the reticulocytes and found the variations to be within the limits of error of the method.

SUMMARY AND CONCLUSIONS

1. The curves for the total number of erythrocytes show waves of several days' duration which are made up of smaller daily variations.

2. The zone within which these fluctuations occur is relatively small.

3. With the methods employed no correlation could be made between points in the curves for the numbers of erythrocytes and those of the cell volumes, hemoglobin contents, corpuscular values and numbers of reticulocytes.

4. Menstruation does not cause any changes in the patterns of the curves. Any fluctuations at that time can only be interpreted as similar to those appearing in the intermenstrual periods.

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EVIDENCE OF THE NON-SEGMENTAL CHARACTER OF SPINAL REFLEXES FROM AN ANALYSIS OF THE CEPHALAD EFFECTS OF SPINAL TRANSECTION (SCHIFF-SHERRINGTON PHENOMENON)[1]

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In previous papers on the cephalad effects of spinal transection (the Schiff-Sherrington phenomenon) we described the reciprocal changes in the reflexes excitability of the fore limbs induced by post-brachial cold-block, novocaine-block, and surgical transection of the spinal cord, namely, *augmentation* of extensor reflexes and *depression* of flexor reflexes (Ruch, 1932; Ruch and Watts, 1934). This phenomenon is one of many in which one part of the segmental nervous system affects the reflex excitability of another part as long as functional connection exists between them. The phenomenon resembles the alterations in hind-limb reflexes following section of the vestibulo- and reticulo-spinal tracts in the decerebrate cat (Fulton, Liddell and Rioch, 1930; Liddell, Matthes, Oldberg and Ruch, 1932) and transection of the spinal cord at various levels, particularly above and below the brachial enlargement. All such instances are open to two interpretations: first, that a transection is effective because it removes an afferent influx (8th nerve and the brachial nerves in the above examples) facilitatory and inhibitory to the limb reflexes; second, that the reflex changes are due to interruption of a portion of the internuncial path (long-circuits) traversing regions of the neural axis at considerable distance from the local afferent influx. In the present experiments the Schiff-Sherrington phenomenon has been utilized to investigate this problem.

Certain observations suggest that the afferent influx to the lumbo-sacral region is the source of the ascending impulses revealed by post-brachial transection. This procedure affects the excitability of the fore limbs when the transection is as low as the third lumbar segment Changes

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in the posture of the hind limbs (Pi-Suñer and Fulton, 1928) and stimulation of hind-limb nerves can inhibit the rigidity of the fore limbs (Sherrington, 1898). Section of certain lumbar roots increases the activity of the knee-jerk (Sherrington, 1898). Other observations, however, suggest that ascending impulses underlying the release of fore-limb extensors by post-brachial transection are wholly independent of the afferent influx into the spinal cord below the level of transection. It was noted in experiments upon hind-limb reflexes, for which the lumbo-sacral segments had been extensively deafferented by nerve section, that nevertheless a low post-brachial transection induced a large increase in the extensor rigidity of the fore limbs. Fulton, Liddell and Rioch (1930) obtained stretch reflexes from quadriceps muscle in acute spinal cats by sectioning the spinal cord just below the roots innervating that muscle. That the limitation of the afferent influx (from the antagonists) was not responsible for the increased reflex activity is suggested by the fact that the stretch reflex is ordinarily absent in the acute spinal cat and difficult to record myographically in chronic spinal animals (Denny-Brown and Liddell, 1927; Matthes and Ruch, 1932) even though the afferent influx to the lumbo-sacral enlargement has been largely eliminated by the nerve sections routinely practised in making mammalian reflex preparations.

In the present experiments we have pursued the suggestion implicit in the above observations, *i.e.*, that the lumbo-sacral afferents play little or no part in the mechanism by which the caudal region of the spinal cord affects the reflex excitability of the fore limbs.

Methods. The preparations used throughout were cats decerebrated at the intercollicular level 2 to 4 hours before the experiment. Reflex contractions to a stretch stimulus of 4 mm. were recorded from the lateral together with the medial head of triceps muscle of the fore limb. The requisite muscle stretch was gained with the "fall-table" device of Liddell and Sherrington (1924); and the response was recorded by a torsion-strip, optically recording, isometric myograph. The triceps muscle was isolated from surrounding soft tissues but retained its nerve and blood supply intact. The nerves of the fore arm were sectioned in the axilla except n. radialis which was sectioned distal to the branches to the triceps group. In all experiments the appropriate dissection was carried up to the point of actual section of spinal roots or tracts before mounting the preparation in the myograph. After control records were secured the transections were made without removing the preparation from its rigid fixations or altering the conditions under which the muscle was contracting. Further details of recording methods, fixation, the stimulating device, etc., are described in a previous paper and by Fulton (1926).

Experimental results. The effect of the following three procedures

upon the decerebrate rigidity and the stretch reflex have been investigated in an effort to determine whether the cephalad effects of spinal transection are due to a removal of the afferent influx over the posterior roots caudal to the transection, or whether the distal segments of the spinal cord exert an influence on the reflexes of more cephalic segments which is independent of the segmental influx: *1*, section of a large number of posterior roots in the lumbo-sacral region; *2*, post-brachial transection after preliminary section of *all* posterior roots below the level of transection; *3*, section of the ascending branches of posterior root fibers in the posterior columns. In connection with the latter experiments some data have been obtained on the path within the spinal cord of the ascending impulses underlying the Schiff-Sherrington phenomenon.

1. *Acute section of posterior roots.* A post-brachial transection at the cephalic end of the lumbar enlargement increases the decerebrate rigidity of the fore limbs. The present experiments were designed to determine whether section of a large share of the posterior roots below this level has a similar effect upon the fore limbs. The stretch reflex of the triceps muscle was recorded myographically for a period immediately before and after section of 8–10 of the large posterior roots which contribute to the lumbo-sacral plexus. This was accomplished with a minimum of excitement of of the unanesthetized preparation and without removing the animal from its rigid fixation or altering the initial tension or other conditions under which the muscle was responding. Before decerebration the spinal cord was exposed by laminectomy and incision of the dura where the long 5–10th post-thoracic roots fan out over the spinal cord. These posterior roots were caught up in 4 to 6 loose ligatures to facilitate section of them in the cramped space of the myograph. Absence of damage to roots was attested by persistence of strong rigidity in the hind limbs. After control records were obtained the roots were sectioned and their proximal ends novocainized to avoid any possibility of discharge from their cut ends (Adrian, 1930). After further records were made, the spinal cord was sectioned at the level of the rostral cut root, the cut faces of the spinal cord were novocainized, and the recording continued. Figure 1 shows a protocol-graph of such an experiment in which the 5–10th (determined at autopsy) post-thoracic roots were sectioned on both sides. No augmentation of the fore-limb stretch reflex was produced by the root sectioning, yet the subsequent transection of the spinal cord increased the stretch reflex more than six-fold. The augmented reactivity persisted without sign of decrease for the hour and a half during which the reflex was periodically recorded. Absence of increase (or a slight decrease) after root section and a strong release by spinal transection were uniformly obtained in three experiments. In one experiment in which the 4–9th post-thoracic roots of

both sides were sectioned the stretch reflex was substantially decreased by root section, from 1.1 kgm. to 0.75 kgm., and increased from 0.75 to 1.45 kgm. by spinal transection.

The 8 or 10 roots sectioned in these experiments include the large posterior roots of the lumbo-sacral region so that the procedure constituted a large and sudden reduction of the afferent influx to the lower lumbar and the sacral region of the spinal cord. Some release of the fore-limb stretch reflex should have been manifested in these experiments if the posterior roots are the source of the inhibition exerted by the caudal segments of the spinal cord upon the extensor reflexes of the fore limbs.

2. *Post-brachial transection after preliminary deafferentation of the lower lying segments.* This series of experiments was undertaken to learn whether the augmentation of fore-limb rigidity, which typically results from a low thoracic spinal transection (Ruch and Watts, 1934), occurs when the spinal

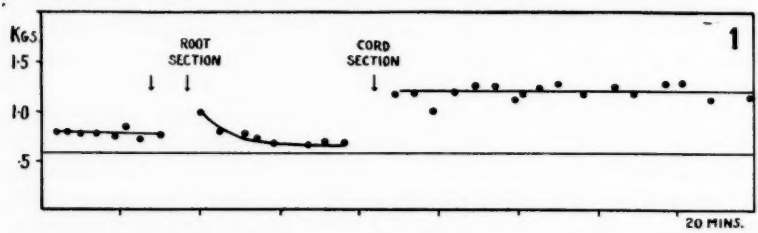

Fig. 1. Stretch reflex of m. triceps in the normal decerebrate cat, after section of the 5–10th post-thoracic posterior roots, and after transection of the spinal cord at the level of entry of the 4th lumbar root; the ordinates are isometric tension; the horizontal line shows the tension exerted by the denervated muscle to the same degree of stretch.

segments below the transection have previously been entirely deprived of posterior root innervation. The day before the experiment *all* posterior roots of both sides below the second or third lumbar segment were sectioned; in some preparations intradurally and in others extradurally. Two animals were prepared by sectioning both anterior and posterior roots. Intercollicular decerebration was performed, the spinal cord was reëxposed, and the preparation was set up in a symmetrical prone position. The rigidity of the fore limbs was then assayed throughout a period before and after transection of the spinal cord at the level of the highest sectioned root by reading from a spring balance the amount of force required to flex the elbow to an angle of 90 degrees.

Observations on brachial rigidity after deafferentation and spinal transection in the lumbo-sacral region: Experiment 1. *Deafferentation of lumbo-sacral segments; decerebration resulting in typical fore-limb rigidity; transection of spinal cord within deafferented area followed by large increase in fore-limb rigidity; healthy male cat.*

Deafferentation (June 7, 1932; 2:30 p.m.). The lumbar enlargement was deafferented, extradurally, on both sides below the third lumbar segment, and the cauda equina was severed. If the division into posterior and anterior roots was not clear, the whole root was severed to ensure complete deafferentation.

Decerebration (June 8, 1932; 10:30 a.m.). The next day after deafferentation, the spinal cord was re-exposed and prepared for transection; the cat was decerebrated at a low inter-collicular level. Hemorrhage and respiratory reaction from decerebration were minimal. The preparation was placed in a symmetrical prone position and the resistance of the elbow to passive flexion to an angle of 90 degrees was measured by a hand balance attached to the wrists.

10:50 a.m. Breathing was slow and regular; pinne and vibrissae and conjunctival reflexes were brisk, but there was no sign of spontaneous twitching of head or neck muscles.

11:10 a.m. The extensor rigidity of the fore limbs and neck was moderate. Measurement of the resistance to passive flexion with the hand-balance gave 0.75 to 1.0 lb. for both left and right arms.

11:50 a.m. The left arm—1.0 lb.; right arm—1.3 lb.

12:22 p.m. The left arm—1.0 lb.; right arm 1.5 lb. The respiration and heart rate rhythms continued to be even and slow. The preparation was in exceptionally good condition.

1:00 p.m. The left arm—1.0 lb.; right arm—1.1 lb. The general condition of the animal was unchanged.

Transection of the spinal cord (June 8). At 1:05 p.m. the spinal cord was cut through cleanly in one snip immediately below the most cephalad of the sectioned roots. The local hemorrhage and the traumatic cardiac and respiratory reaction were slight and transitory. A smart flexion of the fore limbs was the first response to the transection. After 20 to 30 seconds the fore limbs pushed forward slowly into full spastic extension. Periodic observations of the resistance of the limbs to passive flexion were continued.

1:10 p.m. The left arm—2.0 lbs.; right arm—1.8 lb. The increase in rigidity of the fore limbs induced by the transection is unmistakable but the degree of spasticity is not exceptional for an animal after post-brachial transection.

1:29 p.m. Left and right arms—3.0 lbs.; the rigidity of the neck was increased so that head was well supported; respiration and heart were satisfactory.

2:22 p.m. Left and right arm—3.0 lbs. The rigidity was sufficient to support the whole weight of the animal with the fore legs extended in a stilt-like fashion.

3:00 p.m. The triceps muscle was isolated and the preparation mounted in the myograph for recording stretch reflexes; 355 grams of active reflex contraction were recorded during the static period, which was well sustained. The intact limb continued to show a strong extension and was apparently unchanged.

The observations made in this preliminary series of experiments uniformly suggest that deafferentation of the lumbo-sacral segments has no effect detectable by clinical examination upon the decerebrate rigidity of the fore limbs or upon the amount of increase in rigidity which follows post-brachial spinal transection. The same type of experiment was performed with the more exact technique afforded by isometric recording of the stretch reflex. The results of these experiments (see figs. 2 and 3) are in complete agreement with the experiments described above. The excitability of the triceps muscle to a stretch stimulus was not exaggerated

by extensive section of posterior roots in the lumbo-sacral region. On the other hand, spinal transection within the deafferented region increased by several times the reflex response to stretch.

3. *Myographic experiments 3 to 14 days after deafferentation.* The restraining influence exerted by deafferented cord segments upon the fore

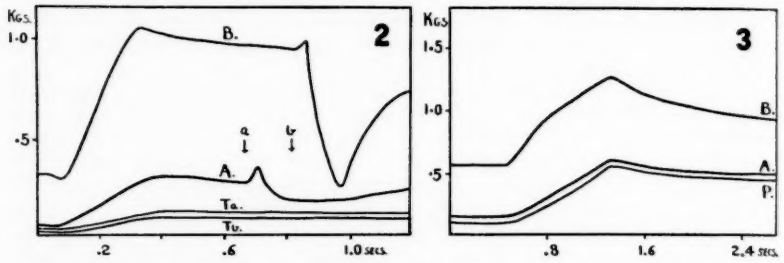

Fig. 2. The stretch reflex of m. triceps: *A*, 20 hours after extra dural section of all posterior roots below L_2 on both sides, and *B*, after spinal transection between L_3 and L_4. *Ta* and *Tb* are signals for the stretch for records *A* and *B*. Arrows *a* and *b* mark the start of repetitive stimulation of the ipsilateral ulnar nerve. The extent of the notch indicates the minimal degree of active reflex response.

Fig. 3. Stretch reflex of m. triceps one day after section of all posterior roots below L_3 on both sides: *A*, and 72 minutes after transection and novocainization of the rostral cut end of the cord, *B*. Curve *P* is the response of the denervated muscle.

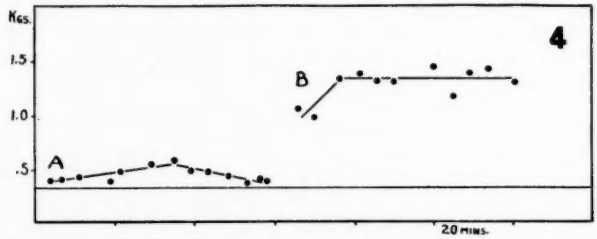

Fig. 4 The stretch reflex of m. triceps after deafferentation and after cord section in the lumbo-sacral region. All posterior roots below the third lumbar segment were sectioned extradurally 13 days before the experiment. Series *A*, after root section, but previous to spinal transection; series *B*, the response to the same stretch after cord section 1 cm. below the 4th lumbar roots. The horizontal line indicates the tension developed by the denervated muscle to the same stretch.

limbs in the above experiments might be thought to be due to a "spontaneous" discharge from the cut ends of the spinal roots. If such occurred, the deafferentation, while complete anatomically, would not constitute "physiological deafferentation." Adrian (1930) has shown that impulses originate from the area of depolarization at the cut ends of sensory nerves,

and that they cease within an hour provided the nerve is bathed in serum and not cooled or desiccated. His observations go far to weaken the ubiquitous criticism of results gained by surgical procedures on the grounds of irritation. In the above experiments, 20 to 24 hours elapsed after sectioning of roots before observations were made, a period which, according to Adrian's data, is sufficient to ensure freedom from this source of error.

The possibility that a "spontaneous" discharge from the sectioned roots was responsible for the activity of deafferented cord segments was directly controlled. For these experiments the deafferentation was performed aseptically 3 to 14 days before the myographic experiment. Each root was sectioned proximal to its ganglion and therefore underwent Wallerian degeneration. Unpublished experiments have shown that 3 days after separation from the ganglion a sensory root no longer elicits a flexion reflex response when stimulated electrically. Figure 4 shows a protocol-graph

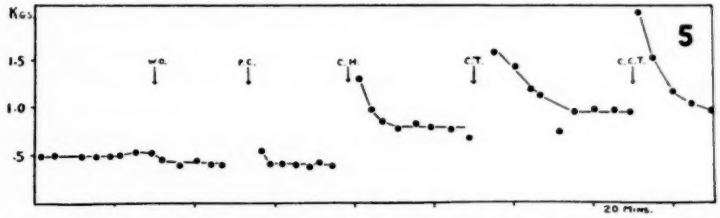

Fig. 5. A protocol-graph showing the effect of partial post-brachial spinal lesions on the fore-limb stretch reflex; *w.o.* = wound opened; *p.c.* = section of posterior columns; *c.h.* = hemisection contralateral to recording muscle; *c.t.* = complete transection; *c.c.t.* = a second, more cephalad complete transection; ordinates = isometric tension of the stretch reflex.

of an experiment performed 13 days after deafferentation. As in the experiments on the 20–24 hour preparations, the deafferentation failed to increase the excitability of the fore-limb stretch reflex, but a large increase (7-fold) followed spinal transection even though only the previously deafferented segments were included below the transection. Since the cut posterior roots were entirely inactive, these experiments, with those previously described, justify the conclusion that the caudal segments of the spinal cord, though devoid of segmental afferent influx, affect the excitability of fore-limb reflexes.

4. *The effect of partial lesions of the spinal cord.* The posterior columns were sectioned bilaterally at a low thoracic level in three experiments with the uniform result that this procedure failed to produce any significant increase in the brachial stretch reflex. An example is shown in figure 5. This result supports the previous experiments in that interruption of ascending limbs posterior root fibers yields the same result as section of the

dorsal roots themselves. In regard to the locus of the fibers underlying the Schiff-Sherrington phenomenon, our experiments at the present stage suggest that they ascend in the ventral half of the spinal cord, probably in the anterolateral columns. This region was found by Fröhlich and Sherrington (1903) to be the only point on the transverse face of the spinal cord from which the decerebrate rigidity of the fore limbs was inhibited by stigmatic stimulation. Experiments in which one lateral half of the spinal cord was sectioned and followed by complete transection suggest that the tracts of each half of the spinal cord innervate both fore limbs. An initial hemisection, whether homolateral or heterolateral to the limb observed, results in a large increase in the tension of the stretch reflex and a further but smaller augmentation follows completion of the transection. Figure 5 shows an experiment in which the reflex tension was increased 374 grams (93 per cent) by a contralateral hemisection, and 183 grams further by completion of the transection. In another experiment, an initial ipsilateral hemisection increased the tension of the fore-limb stretch reflex by 394 grams and completion of the transection added a further 291 grams. No systematic difference in the resistance of the two limbs to passive flexion has been detected from a lateral hemisection. These observations, while limited, suggest that the ascending paths of one lateral half of the spinal cord act bilaterally and approximately to the same extent upon the two fore limbs.

Discussion. The lumbo-sacral segments of the spinal cord are in some way involved in the reflex activities of the fore limbs. When the influence of these segments is removed by post-brachial transection there is an immediate and strong increase in the decerebrate rigidity and in the stretch reflex of the fore limbs. The phenomenon offers many parallels to the inhibitory action of the descending tracts (bulbo-spinal) of the anterior columns on the flexion reflex of the hind limbs (Liddell, Matthes, Oldberg and Ruch, 1932). But that the lumbar enlargement is not the site of an afferent influx (analogous to the 8th nerve) which continuously inhibits fore-limb extensors is suggested by the present experiments. Acute section of a large share of the posterior roots of the lumbar and sacral region is without augmentatory effect upon the fore-limb stretch reflex. Chronic section of *all* sensory roots below the third lumbar segment is likewise without effect. A spinal transection which removes only previously deafferented segments greatly augments the extensor responses of the fore limbs. This critical observation shows that the inhibitory influence of the caudal segments is not derived from their segmental afferent influx.

The lumbo-sacral segments, though completely deprived of posterior root innervation, either *originate* or *transmit* a rostrally directed stream of impulses which decrease the reflex activity of fore-limb extensor muscles. The first and more probable alternative implies that caudad segments

transmit impulses from intact bulbar and spinal roots (particularly the roots supplying *m. triceps*) to brachial motoneurones and therefore are significant as a field for the irradiation of impulses originating higher in the nervous system. The distal segments may contain "delay paths" or "reverberating circuits" such as those postulated by Forbes (1922, 1929), Ranson and Hinsey (1930) and Lorente de Nó. A difficulty for this interpretation is that both bulbo-spinal impulses and the afferent discharge from the stretched muscle are predominantly excitatory to extensor muscles, while the ascending impulses in question are strongly inhibitory to reflex extension. Fulton (1926) suggested that certain descending tracts act upon the internuncial neurones of spinal reflex arcs. If descending tracts of the decerebrate animal end in part upon internuncial neurones (Rasdolsky, 1923) which ascend to the brachial enlargement, a potential circuit is formed which would remain intact after complete deafferentation of the lumbar enlargement.

An alternative to the above "long-circuiting" mechanisms is that the spinal cord originates impulses in the absence of afferent innervation. While "automaticity" is a radical, and to many, a repugnant concept, it is not entirely without support (Barcroft, 1919; Adrian, 1932). The respiratory center maintains rhythmic discharge after considerable reduction of afferent sources (Stewart, 1907; Stewart and Pike, 1907a, b; Winterstein, 1911). Similarly, according to Graham Brown (1911, 1913, 1914) spinal progression is largely independent of afferent stimulation. Narcosis progression occurs after the narcotic has largely or entirely blocked reflex response to strong electrical stimulation of limb afferents (Graham Brown, 1914; Storm van Leeuwen, 1914). A portion of the spinal cord isolated by spinal and posterior root section discharges in response to strychnine and asphyxia (Sherrington, 1898). Adrian (1931) and Adrian and Buytendijk (1931) have shown that the central ganglia of the gold-fish and the water-beetle exhibit rhythmic potential changes coincident with a discharge of efferent impulses, which persist after section of all afferent nerves.

Since these experiments involved narcosis or interference with the circulation or respiration, they perhaps illustrate no more than the tendency of nerve cells to discharge when asphyxiated. The critical experiment which will rule out interpretation on the basis of automatic spinal cord function has not been devised. It is therefore still possible that the impulses from deafferented caudal segments which affect the excitability of the fore limbs are a primitive intrinsic discharge which is controlled rather than initiated by hind-limb afferents.

The failure of deafferentation in the lumbo-sacral region to annul the cephalad effects of spinal transection in that region is not easily reconciled with the conventional picture of segmental reflex arcs. The experiments reported above and in a previous paper (Ruch and Watts, 1934) demon-

strate that the spinal cord functions to some degree as a unit, *i.e.*, that the brachial stretch reflex is not determined alone by segmental reflex arcs or by these and *direct* descending tracts from the brain stem; that distal and remote spinal segments are in some way involved in determining the excitability of fore-limb reflexes; and that this activity is not derived from the posterior root influx to the caudal segments of the spinal cord.

SUMMARY

Although spinal transection in the upper lumbar region of a decerebrate cat induces an extreme hyperreflexia of the fore-limb extensor muscles, acute section of a large share of the posterior root fibers of the lumbo-sacral enlargement or section of the posterior columns is without cephalad effect.

Section of *all* lumbo-sacral dorsal roots does not augment fore-limb extension, nor diminish the effect of a subsequent post-brachial transection. A transection which eliminates only previously deafferented cord segments augments the decerebrate rigidity and the stretch reflex of brachial extensor muscles. The inhibitory action exerted by lumbo-sacral segments on the fore limbs is subserved by some intra-spinal system of neurones, which operates in the absence of segmental afferent influx to the lumbar enlargement.

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THE GLYCOGEN CONTENT OF LIVER AND MUSCLE IN THE COMPLETELY HYPOPHYSECTOMIZED DOG[1]

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To judge from the changes produced in 3 blood constituents, namely, the rise in glucose and lactic acid and the fall in inorganic phosphorus, the completely hypophysectomized dog is capable of responding to epinephrine (1). This response, however, is less than in the normal animal. Since this decrease was observed as late as 4 months after excision of the gland, it may be concluded that in the absence of hypophyseal hormones epinephrine does not exert its normal effects on carbohydrate metabolism. Although it accelerates the conversion of liver glycogen to blood sugar, the Coris (2, 3) have shown that the amount of glycogen normally present in the liver will not account fully for the glucose thrown into the blood stream by epinephrine. They first demonstrated that epinephrine in suitable doses effects a redistribution of glycogen between liver and muscle by releasing lactic acid from the latter and then reconverting it into glycogen in the liver, a cycle that makes muscle glycogen available for blood sugar purposes. In attempting to explain the decreased epinephrine response of the completely hypophysectomized dog, attention was therefore directed to the glycogen content of both liver and muscle. Although glycogen values for hypophysectomized animals have been previously reported (4–6), the irregularity of the results obtained has made desirable the determination of the glycogen content of the liver and muscles of dogs maintained in the same nutritional states in which the abnormal epinephrine reactions were found.

EXPERIMENTAL. Fully grown female dogs were used in this investigation. During their entire stay in the laboratory both normal and hypophysectomized dogs were kept under identical environmental and dietary conditions. The diets employed have been noted elsewhere (7). The dogs were fed twice daily at 8:00 a.m. and 4:00 p.m. Muscle and liver were removed for glycogen determinations between 9:00 and 10:00 a.m.,

[1] The expense of this investigation was defrayed in part by a grant to one of us (I. L. C.) from the Research Board of the University of California, Berkeley.

and prior to this the dogs had received their last meal at 4:00 p.m. of the previous day. Thus, at the time glycogen estimations were made, a period of 17 to 18 hours had elapsed since the last ingestion of food

Hypophysectomized dogs. The operative manipulations involved in the removal of the pituitary gland by the intracranial route have been previously described (7, 8). The hypophysectomized dogs were placed on the experimental diet on the day following the operation and with the exception of the first week they possessed good appetites, ingesting all food soon after it was served.

Normal dogs. The 8 normal dogs studied were maintained on the same diet for 3 to 5 weeks prior to removal of tissues for glycogen estimations.

Preparation of dogs. Anesthesia was induced by the intraperitoneal injection of 50 mgm. of amytal (in the form of its sodium salt) per kilo of

TABLE 1

The glycogen content of liver and muscle of normal female dogs

(All values are given in grams of fermentable sugar per 100 grams of tissue)

DOG	WEIGHT	LIVER GLYCOGEN	MUSCLE GLYCOGEN	
			Right leg	Left leg
	kgm.			
N21	11.4	5.47	1.02	0.97
N22	8.3	4.58	0.81	0.84
N23	7.9	7.77	0.96	0.97
N25	9.1	8.33	1.32	1.31
N26	7.8	6.61	1.11	1.04
N27	6.2	9.03	1.29	1.33
N24	8.5		0.91	0.87
N20	9.3	5.92		

body weight. The skin of both hind limbs was dissected free from the subcutaneous and muscle tissues, care being taken to avoid excessive bleeding. The muscles were then rapidly surrounded with a slush prepared from carbon dioxide powder and ether and kept frozen solid until removed for sampling. The liver was then rapidly excised.

Sampling of liver. The surface of the liver was wiped free of adhering blood, and samples were obtained by punching various areas with a brass tube built like a cork borer with an inside diameter of 6 mm. Approximately 25 samples of liver, each measuring 1 to 3 cm. long and weighing approximately 0.5 gram, were rapidly removed from the different lobes and placed in 6 pyrex test tubes containing 30 per cent potassium hydroxide. Each tube received a total of 1.5 to 2.5 grams of liver tissue. The time between excision of the liver from the peritoneal cavity and immersion of the samples in potassium hydroxide occupied about 2 minutes. The

value recorded for the liver glycogen of each dog represents the average of 6 closely agreeing determinations.

Sampling of muscle. The frozen limb was removed from the body and immediately thereafter immersed in liquid air. It was then cut into small blocks which were kept in liquid air until removed for dissection. By means of a sharp hatchet the frozen blocks of tissue were carefully dissected, pieces of muscle being freed from visible adhering connective tissue and fat. As the pieces of muscle were obtained they were returned to liquid air, and when the whole limb had been dissected the muscle thus obtained was passed through an electrically driven meat grinder kept cold by surrounding the outside with a slush of carbon dioxide snow and ether. The muscle

TABLE 2

*The glycogen content of liver and muscle of completely hypophysectomized female dogs**†

(All values are given in grams of fermentable sugar per 100 grams of tissue)

DOG	WEIGHT	INTERVAL SINCE HYPOPHYSECTOMY	LIVER GLYCOGEN	MUSCLE GLYCOGEN	
				Right leg	Left leg
	kgm.	*months*			
H15	14.0	4.2	10.10	0.97	0.97
H16	13.7	3	5.46	0.90	0.86
H17	8.2	3	9.87	0.72	0.72
H18	15.8	2.5	8.44	0.71	
H19	9.0	4	10.45	0.82	0.89

* In the 5 dogs of this group, serial sections were made of the bases of the brains and the whole hypophyseal regions carefully examined. There was a complete absence in all of these animals of anterior, posterior and intermediate lobes of the gland as well as of all stalk tissue. Microscopic remnants of the pars tuberalis were found in dogs H15, H17, H18 and H19, whereas H16 showed a complete absence of this structure.

† The thyroids of these 5 dogs contained flattened epithelium and follicles well filled with colloid.

was then thoroughly pulverized in a chilled iron mortar. Liquid air was used freely to keep the tissue in a frozen state. Four samples of 5 grams each of pulverized muscle were taken from each limb for glycogen determinations. The value recorded for the glycogen content of each leg represents the average of 4 closely agreeing determinations.

Since it is well known that the different muscles of the same limb contain variable amounts of glycogen, it has become customary to determine the glycogen content of a few carefully dissected muscles of each limb. While such a method may afford a means of comparing the glycogen content of the muscles of the 2 limbs of the same animal, it does not provide so satisfactory a means of comparing the amount of glycogen in the muscles of

different animals. The method adopted in the present investigation furnishes a sample obtained from all the muscles of the hind limb; hence such a sample may be considered as more representative of the musculature of the body than that obtained from a few isolated muscles.

Glycogen determination. Glycogen was determined by the Pflüger method as modified by Good, Kramer and Somogyi (9). The further modification of Cori and Cori (10) in which the glycogen was dissolved in water and reprecipitated was adopted. The reducing power of the glycogen hydrolysate was determined before and after yeast fermentation. The yeast was prepared and washed according to Somogyi's method (11). The improved copper reagent of Shaffer and Somogyi (12) was used for sugar determination. Glycogen is expressed as grams of fermentable sugar per 100 grams of tissue.

RESULTS AND DISCUSSION. The glycogen values for the livers of 8 normal dogs ranged between 4.6 and 9.0 per cent, while the highest and lowest values for muscle glycogen were 0.81 and 1.3 per cent. No decrease in the glycogen content of liver and muscle was found in the 5 completely hypophysectomized dogs that were maintained in nutritional states comparable with that of the normal animals. Thus the lowest value for liver glycogen in the hypophysectomized dogs was 5.5 per cent and the highest well over 10 per cent. The muscles of these dogs contained between 0.71 and 0.97 per cent glycogen.

The results of the present investigation show that in the complete absence of hypophyseal hormones for as long as 4 months there is no interference in the ability of the liver and muscle to store glycogen. It is indeed a peculiar paradox that the hypophysectomized dog may contain as much as 10 per cent glycogen in the liver and as much as 1 per cent in the muscles at the same time that it displays an exaggerated response to insulin (7) and a diminished response to epinephrine (1). Whatever the defect in the carbohydrate metabolism of this animal may be, *the present study makes it clear that its abnormal epinephrine and insulin reactions cannot be ascribed to a deficient storage of glycogen in the tissues.* Because of these large carbohydrate reserves it would seem—as already pointed out by Corkill, Marks and White (5)—that in the absence of hypophyseal hormones there is an interference in the mechanism whereby glycogen from muscle and liver is made available for blood sugar purposes. On the other hand, it is also conceivable that the increased insulin response and the decreased epinephrine response may be due to the fact that the injection of these hormones leads to an acceleration in the uptake or in the utilization of carbohydrate by the tissues of the hypophysectomized dog, but this seems unlikely in view of the depressed thyroid activity that follows excision of the hypophysis.

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THE CARDIO-VASCULAR RESPONSES OF PRE-ADOLESCENT BOYS TO MUSCULAR ACTIVITY[1]

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This study extended over a period of two years and was undertaken with the object of securing data which would give a picture of the cardio-vascular reactions of a group of pre-adolescent boys to various amounts of muscular exercise. The work began with a group of five-grammar school boys ranging in age from 9 to 13 years. The youngest and the oldest boys, respectively, were used only for the early determinations; since the former moved away from town and the latter, just entering puberty, was so much larger as soon to be out of the class of the other boys.

The physical measurements of the group were as follows:

	DATE	HEIGHT	WEIGHT
			lbs.
R. B.	Mar. 9, 1932	5′½″	80
	Nov. 4, 1932	5′3″	87
	Apr. 8, 1934	5′9″	116
J. B.	Dec. 12, 1931	5′3″	90
	Sept. 30, 1932	5′4¾″	96
	Apr. 8, 1934	5′10″	120
J. H.	Mar. 22, 1932	5′¾″	103
	Oct. 29, 1932	5′2″	109
	Apr. 8, 1934	5′7¼″	131
H. C.	Dec. 9, 1931	4′8½″	80
K. V.	Mar. 1, 1932	5′8¼″	131

The three subjects who served through the two years were growing rapidly. All three entered puberty in the autumn of 1933 and the only data collected from them after that time was the cardiac output. Marked differences in physical ability among the boys are evident from our experi-

[1] The expense of this investigation has been met by a grant from the Charles Himrod Denison Fund.

mental results. R. B. is the younger brother of J. B. Both boys were healthy and active, but R. B. was noticeably less strong than his older brother and was subject to frequent colds.

Postural responses. During the spring of 1933 the boys were visited on two different mornings, while still in bed, for the recording of basal blood pressure and pulse rate. These data are given in table 1.

The early morning data here given correspond closely to those of earlier students of prepubescents. McCurdy found that the systolic pressure in recumbency of prepubescents ranged between 103 and 111 mm. Hg. Wiggers observed the mean values of the systolic and diastolic pressures to be 100 and 70 mm. at 10 years and 110 and 75 mm., respectively, at 14 years of age. The pulse rate at 12 years is said to range from 70 to 76 per minute. Wiggers finds that it fluctuates around 85 by the 12th year and around 70 after puberty.

TABLE 1

Before rising in the morning, March 1933

	BLOOD PRESSURE IN MM. Hg									PULSE RATE PER MINUTE		
	Systolic			Diastolic			Pulse pressure					
	R. B.	J. B.	J. H.	R. B.	J. B.	J. H.	R. B.	J. B.	J. H.	R. B.	J. B.	J. H.
1st morning...	103	103	99	79	77	74	24	26	25	72	68	67
2nd morning...	104	104	98	77	78	69	27	26	29	90*	68	72

* The high pulse rate recorded for R.B. on the second morning was probably due to a cold from which he was suffering at the time.

The basal pulse rate in our boys was more rapid than it ordinarily is in young men of college age. The lowest rate obtained on these boys was 67 beats per minute, while in young men the rate is frequently as low as 45 and is rarely as high as 65.

The boys were also examined mid-afternoon. Each lay down for 15 minutes two hours or more after the noon meal. Invariably their arterial blood pressures were lower and their pulse rates were higher than during the basal condition of early morning. In adults the afternoon arterial pressure is generally higher than in the early morning. The average afternoon systolic pressures of the boys were J. B. 98, R. B. 100, and J. H. 90; the average diastolic pressures were 69, 72, and 66 respectively; while the pulse rates were 76, 80, and 82 respectively.

During standing the pulse rate of these boys averaged higher than it commonly does in young men. In college men, who lead as active a life physically as did our boys, the standing pulse rate averages around 90; while in the boys it ranged between 96 and 116. The increase in the fre-

quency of the heart beat when the standing posture was taken after recumbency ranged from 20 to 34 beats. In healthy adults this difference ordinarily ranges from 6 to 15 beats.

The systolic blood pressure invariably rose in our boys when they took the standing position after a period of recumbency. In a study of 2000 young men Schneider and Truesdell found that only 55.6 per cent had their highest systolic pressure in the erect position, while 26.5 per cent had it during recumbency. Our boys experienced increases of from 4 to 15 mm. Hg, which were within the range of healthy young men who experience a rise in this pressure on standing. The diastolic pressure of the boys always rose when they stood up, the increase ranging from 12 to 20 mm. Schneider and Truesdell found the average rise in young men to be 8 mm.

The most marked difference in the blood pressures of these boys and of healthy young men is in the pulse pressure. Schneider and Truesdell found for 2000 men a mean recumbency pulse pressure of 47 mm., with

TABLE 2

Prolonged standing, R. B.

TIME	PULSE RATE	BLOOD PRESSURES IN MM. Hg		
		Systolic	Diastolic	Pulse pressure
Rest	78	98	72	26
3′	96	105	88	17
6′	102	105	88	17
9′	100	105	88	17
12′	100	106	90	16
15′	100	112	104	8

two-thirds of all cases between 38 and 57 mm.; while in standing the mean was 42 mm., with two-thirds between 31 and 53 mm. In our boys the recumbency pulse pressure ranged from 25 to 29 mm. and the standing between 20 and 22 mm. These data indicate that the output of the heart per beat was decidedly less in boys than in men. For the 2000 men Schneider and Truesdell found that the pulse pressure averaged a fall of 6 mm. when the change was made from recumbency to the standing position. In our boys this fall was about the same, ranging from 5 to 8 mm.

Turner's test of physical efficiency was applied to the boys. The test, which depends on cardio-vascular reactions during a prolonged period of standing absolutely still for 15 minutes, was unsuitable for our subjects. The boys found the long standing intolerable. All were on the verge of fainting before the period ended. Even practice failed to make them comfortable throughout the entire 15 minutes. A conspicuous sign of the onset of difficulty was sweating, which was sometimes profuse. The

longest J. B. managed to stand was 9 minutes, while he once fainted at 6 minutes. R. B. experienced the least trouble, but as will be seen in table 2 he was in real difficulty at the 15th minute. At this time both his systolic and diastolic pressures were rising, with the diastolic rising at the greater rate. This caused a rapid fall in the pulse pressure and foretold fainting.

The pulse pressure of each boy always fell, ordinarily progressively throughout the period of standing, and usually as the diastolic pressure rose. Occasionally the heart rate accelerated inordinately, as in the case of J. H. when it reached 136 beats per minute. His recumbent pulse rate in this experiment was 84 and when first taking the standing position his rate was 116, where it remained fairly constant until the twelfth minute, after which symptoms of discomfort rapidly developed and the boy became faint.

Pulse rate after mild exertion. The pulse rate was observed after the moderate exertion of stepping up on a chair, 18 inches high, 5 times in 15 seconds. Flack and Bowdler conclude for young men that after this form of exercise an increased rate of over 25 and a return period of over 30 seconds are indicative of poor physical condition. Schneider and Truesdell in a study of 204 physically fit aviators obtained a mean increase of 12 beats after this exercise, with 4 per cent showing an increase of 25 or more beats; while 8 per cent experienced no acceleration of the pulse. Our boys immediately after this amount of effort gave accelerations of from 10 to 20 beats and always made the return to normal rate within 60 seconds. The most prompt recovery was made in 30 seconds, while the normal time was within 45 seconds. In the group of aviators studied by Schneider and Truesdell only 93 per cent were back to normal in 60 seconds. We may conclude, therefore, that the pulse rate increase and recovery of our boys was comparable to the responses of healthy young men to this form of exercise.

Pulse rate during short work periods of graded intensity. Work was performed on a Krogh bicycle ergometer which was calibrated in foot-pounds of work at a speed of 80 revolutions of the pedals per minute. The periods of exercise were five minutes in duration and of increasing severity. The observations at the various loads of work were made after three minutes of "warming up." In the case of the heaviest load the "warming up" period was somewhat shortened if the strength of the boy was being overtaxed. Fifteen minute rest intervals were ordinarily taken after each work period; or when the heart failed to return to its pre-exercise rate within that time, a longer rest was allowed.

We determined the pulse frequency in all five of our subjects and give their average responses in table 3. Similar experiments (Schneider) made with adult males have shown that the frequency of the heart beat will maintain an approximate linear relationship with the demands of

physical work up to a certain load, which varies with the individual; and that beyond this, when heavier loads are undertaken, the heart will accelerate less per unit of work than it did prior to that load. Eventually a still greater load may be undertaken to which the heart is unable to respond with any further increase in frequency of beating.

The linear relationship between frequency of heart beat and load of work was somewhat obscured for the boys by the inordinate acceleration of the heart during the lightest load, 1500 ft. lbs. In only J. B. was this increase in the pulse moderate; in the other four boys it ranged from 10 to 20 beats more than was to be expected. This seems to bear out the observation that the human machine, like man-made machines, works the least efficiently when idling or working under only slight stress. K. V., who was just entering puberty, gives a reasonably good illustration of the linear relationship. By averaging his increases for loads of 1500 and 3000 ft. lbs., we found he had an augmentation of 25 heart beats for each step-up

TABLE 3

	FREQUENCY OF HEART BEAT PER MINUTE					PULSE INCREASE			
	Rest	1500 ft. lbs.	3000 ft. lbs	4500 ft. lbs.	6000 ft. lbs.	1500 ft. lbs.	3000 ft. lbs.	4500 ft. lbs.	6000 ft. lbs.
R. B.	107	141	165	192	197	34	24	27	5
J. B.	104	129	158	174	187	25	29	16	13
J. H.	104	142	162	174	182	38	20	22	8
H. C.	104	135	152	169	179	31	17	17	10
K. V.	78	111	128	155	178	33	17	27	23

in the load of work. For him a load of 6000 ft. lbs. was not an overload as it was for the other boys. For R. B. and J. H. a load of 6000 ft. lbs. was clearly an overload; hence, when this load was carried, the increment of acceleration for the step-up in load for R. B. was only 5 beats instead of 27, as it should have been with a normal load; and for J. H., 8 instead of 27 beats.

Differences in physical condition among the boys reveal themselves in several respects when the exertion pulse rates are considered. Among the four pre-adolescents, H. C., the youngest, meets the requirements of exertion with a smaller demand on the heart than the others; even with a load of 6000 ft. lbs. his response is the more nearly in linear proportion. R. B., who throughout this study was weaker than his brother, J. B., probably reached the maximum efficiency of heart beat with a load of 3000 ft. lbs. It is generally conceded that the efficiency of the heart begins to progressively fall off at rates between 160 and 180 beats per minute, since at a higher rate the diastolic period of the heart is too brief to permit com-

plete filling. R. B.'s heart attained a frequency of 165 beats with a load of 3000 ft. lbs. and of 192 with the next larger load. H. C., the youngest boy of the group, fared better and probably only reached the upper limit of maintained efficiency as he carried a load of 6000 ft. lbs., when his heart rate reached 179 beats per minute. K. V. illustrates the advantage gained by adolescence, even in its early stage, in that his heart rate was slower than that of the other boys both at rest and during work. Apparently, because of this advantage, he was able to maintain the linear relationship between the load of work and frequency of heart beat with each added increment of work up to and including 6000 ft. lbs.

Oxygen pulse. This is defined as the amount of oxygen consumed by the body from the blood of one systolic discharge of the heart. Its value is calculated by dividing the amount of oxygen that is absorbed by the individual per minute by the number of heart beats in a minute. An increase in the size of the oxygen pulse may be indicative of an augmented stroke volume of the heart or of a larger unloading of oxygen from the

TABLE 4

Oxygen pulse in cubic centimeters

SUBJECT	REST	1500 FT. LBS.	3000 FT LBS.	4500 FT. LBS.	6000 FT. LBS.
R. B.	2.2	5.4	6.1	6.8	7.3
J. B.	3.0	6.8	7.1	8.7	9.4
J. H.	2.9	6.6	8.2	8.7	9.8
H. C.	2.9	5.7	7.2	8.2	9.2
K. V.	4.5	9.1	10.7	12.0	13.6

blood as it flows through tissue capillaries. A summary of the oxygen pulse data obtained from the boys is given in table 4.

Henderson and Prince found that the oxygen pulse increases rapidly with acceleration of the heart, and in most cases reaches its maximal value of 11 to 17 cc. at heart rates of 130 to 140 beats per minute; while with further acceleration it may tend to decrease. In our pre-adolescent boys the oxygen pulse was constantly less than in young men. In a study of 5 men at rest Schneider (1931) found that the oxygen pulse ranged from 3 to 4 cc. (average 3.4 cc.) and that while carrying a load of 6000 ft. lbs. on the bicycle ergometer it ranged from 11.7 to 12.8 cc. (average 12.3 cc.). For the pre-adolescent boys the oxygen pulse at rest ranged from 2.2 to 3 cc. and with a load of 6000 ft. lbs., from 7.3 to 9.8 cc. R. B., our weakest subject, had the smallest oxygen pulse, which was only 2.2 cc. during rest and 7.3 cc. while carrying a load of 6000 ft. lbs. At no time did the pre-adolescent boys reach the maximal values for men obtained by Henderson and Prince.

It is interesting to find that K. V., who had just become adolescent, had an oxygen pulse during rest of 4.5 cc. and while carrying a load of 6000 ft. lbs. one of 13.6 cc. These are slightly larger than those observed in adult men by Schneider.

Pulse rate during prolonged periods of work. In these experiments the subject, as a rule, proceeded until he felt he could continue no longer. Bowen found that during riding on a stationary bicycle for about 30 minutes there occurred a sudden and rapid primary rise in the pulse rate when work was begun, that this was followed by a more gradual secondary rise, and that the maximum rate was often reached in from 3 to 5 minutes. Only three of the adolescent boys were studied during prolonged work. The response to moderate loads was similar to that made by young men. With a load of 3000 ft. lbs. the responses were similar to some obtained by Bowen, the primary rise occurring in R. B. and J. H. in the first minute, and in J. B. in 3 minutes; while the secondary rise continued climbing for some time, reaching its maximum in R. B. in 5 minutes, in J. B. in 20 minutes, and in J. H. in 25 minutes, after which each boy maintained a fairly steady state.

With heavier loads the frequency of heart beat became excessive and accelerated more rapidly than it ordinarily does in adults. With a load of 4500 ft. lbs. the pulse rate of J. H. rose from 94 to 135 during the first minute, to 148 by the fifth minute, and to 164 by the seventeenth minute, where it remained until the end, 25 minutes. J. B. made a similar response, reaching a rate of 190 by the twenty-fifth minute, where it remained until the thirty-fifth minute when he felt exhausted. R. B.'s pulse continued to accelerate throughout a work period of 10 minutes to the unusual rate of 210 beats per minute.

With a load of 6000 ft. lbs. the pulse of each of the three boys failed to reach a plateau; in J. B. it rose steadily, after the primary rise, to 192 in 15 minutes; in J. H., to 196 in 10 minutes; and in R. B., to 226 beats in 6 minutes. Each boy had worked until his legs gave out.

In this type of work R. B. showed his inferiority in each experiment by the extraordinary rates reached by his heart.

Arterial blood pressure during work of graded intensity. In these experiments the boys mounted the bicycle ergometer after 20 minutes of rest and there again rested for about two minutes before the pre-exercise pressures were taken. Then, as the experiment proceeded, a "warming up" period of three minutes preceded the determination of the blood pressures. Several readings were taken before the exertion was discontinued. Following each work period the boys rested until their pressures had returned to normal and then proceeded with the next higher load. The averages for three sets of experiments on each boy are given in table 5.

The systolic pressure rose in each boy with every increment in the load

of work. No uniformity in rise was evident although the loads were increased by equal units. The extent of the rise in no way corresponds to the known physical condition of the boys and in no case was the degree of rise unusual.

In adults the diastolic pressure ordinarily shows a rise during physical exertion, but occasionally the systolic and diastolic pressures may be affected in opposite directions. In our boys the diastolic pressure, almost without exception, fell as the load of work was increased. Here, as was true of the systolic pressure, there was no proportional relationship between the changes in load and in pressure. Apparently the fall in diastolic pressure was not dependent on the heart frequency. No attempt was made to associate it with an unusual dilatation of peripheral vessels which might account for the drop in pressure.

As is usually the case in physical exertion the changes in pulse pressure more closely follow the fluctuations of the systolic than of the diastolic

TABLE 5

Arterial blood pressures in mm. Hg

LOAD	SYSTOLIC			DIASTOLIC			PULSE PRESSURES		
	R. B.	J. B.	J. H.	R. B.	J. B.	J. H.	R. B.	J. B.	J. H
Rest	108	106	107	62	70	72	46	36	35
1500 ft. lbs.	115	118	130	66	68	71	49	50	59
3000 ft. lbs.	133	121	137	59	65	67	74	56	70
4500 ft. lbs.	147	131	151	57	62	63	90	69	88
6000 ft. lbs.	150	140	164	58	62	61	92	78	103

pressure. In these boys the drop in diastolic pressure caused the increases in pulse pressure to be unusually large for the increment of work performed. If the pulse pressure were a reliable index of the heart stroke we should expect that the output of blood per beat by the heart would be augmented more than it ordinarily is by physical exertion. Our study of the output of the heart, which follows, fails to support this possibility.

Output of the heart. For these determinations Christensen's modification of Grollman's acetylene method was used. Unfortunately these experiments were the last made on the boys and occurred after each of them had entered adolescence. The summaries of the data are given in table 6. The consumption of oxygen while the boys were at rest on the bicycle ergometer was within the range found for adult men. The output of the heart per minute during rest was also about the same as occurs in men, when sitting quietly on a bicycle. This is usually slightly above that of complete rest since this position does not permit of complete relaxation. The minute volumes per square meter of body surface for the boys were R. B. 2.7, J. B. 3.1, and J. H. 2.8 liters. These are within the basal range as found by Marshall and Grollman.

The data for a load of 1500 ft. lbs. show, as did the study of the frequency of the heart beat, that there was a large amount of overwork in performing the light loads. Thus in carrying the load of 1500 ft. lbs. the minute volume output of the heart increased in R. B. by 120 per cent, in J. B. by 84.6 per cent, and in J. H. by 170 per cent. When the next larger load was carried the increases were only 50, 21, and 0 per cent respectively.

While the output of the heart per beat when the boy was at rest was not very different from that of an adult, nevertheless during work the stroke volume was clearly less than in the adult. Christensen found that in the untrained adult female a load of approximately 6000 ft. lbs. brought about a stroke volume of 120 cc., while in a trained male it might be as large as 140 cc. Our boys, while carrying a load of 6000 ft. lbs., had stroke volumes that ranged between 95 and 109 cc.

TABLE 6

SUBJECT	OXYGEN CONSUMPTION, ML.				ARTERIO-VENOUS O_2 DIFFERENCE, ML. PER LITER OF BLOOD			
	Rest on ergometer	1500 ft. lbs.	3000 ft. lbs.	6000 ft. lbs.	Rest on ergometer	1500 ft. lbs.	3000 ft. lbs.	6000 ft. lbs.
R. B.	305	900	1200	1860	69	93	101	115
J. B.	350	800	1080	1870	68	83	101	123
J. H.	300	1050	1250	1640	63	83	100	105
	CARDIAC OUTPUT LITERS PER MINUTE				SYSTOLIC OUTPUT, ML.			
R. B.	4.4	9.7	11.9	16.2	51	73	82	99
J. B.	5.2	9.6	10.7	15.2	63	80	86	95
J. H.	4.7	12.7	12.5	15.6	55	106	102	109

The output of the heart per minute was also below the quantities found for adults by Christensen when similar loads of work were accomplished, but the differences were not proportionately as large as for the stroke volume. The greater frequency of heart beat in the boys accounts, at least in part, for this fact. Christensen's data show the cardiac output per minute for a load of 6000 ft. lbs. to be 18 or 19 liters of blood. In our boys this load caused an output of from 15.2 to 16.2 liters per minute.

SUMMARY

Basal arterial pressures were rather low—98 to 104 mm. Hg. Mid-afternoon pressures were still lower. Among adults these pressures are usually higher in the afternoon than in the morning. The basal pulse rates were more rapid than in adults. During standing the pulse rate took a higher tempo than is common among adults. The change from recum-

bency to standing accelerated the pulse as much as 20 to 34 beats, while among adults the acceleration ordinarily ranges from 6 to 15 beats.

In changing from the recumbent to the standing posture the diastolic arterial pressure invariably rose more than it does in adults. The pulse pressure was uniformly less than in adults.

Prolonged quiet standing always brought on symptoms of fainting. Noticeable changes were a rapid fall in pulse pressure and an inordinate acceleration of the frequency of the heart beat.

The pulse response to the moderate exercise of stepping up on a chair 5 times in 15 seconds was no greater than occurs in men.

When subjected to short periods of work of graded intensity the pre-adolescent failed to show the linear relationship between load of work and pulse rate that occurs among adults. A boy who had just entered pubescence gave a good illustration of this linear relationship.

For all of the pre-adolescents 6000 ft. lbs. was clearly an overload of work.

The oxygen pulse was uniformly less both during rest and exertion than it is among adults.

During periods of prolonged work the heart frequency response to moderate exertion was not noticeably different from that of men, but with loads of 4500 and 6000 ft. lbs. the heart either was slow in reaching a steady state or failed to reach it and the acceleration was excessive.

The only unusual feature of arterial blood pressure responses was a drop in the diastolic pressure during the periods of exertion.

The output of blood by the heart per beat during periods of rest was within the range common to men, but during periods of work it was distinctly less than for men.

The minute volume output of the heart was also less among the boys than among men but not proportionately the same as that of the stroke volume. The greater frequency of the heart beat tended to offset the smaller stroke volume.

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THE INFLUENCE OF DIVIDED DOSAGE OF GONADOTROPIC EXTRACTS IN THE IMMATURE MALE RAT

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It was recently shown in this laboratory (1) that divided dosage of or the addition of suitable protein precipitants to pituitary gonadotropic extracts administered to immature female rats produced an augmentation of ovarian weight increment and modified the response, so that follicular ovaries were obtained with preparations which, given in the ordinary way, produced luteal bodies. Prolan differed from the pituitary preparation in that no augmentation effect was produced.

The present report is an extension of these studies to the immature male rat, using tannic acid as the protein precipitant.

EXPERIMENTAL. The pituitary gonadotropic powder was prepared in this laboratory according to the method of Wallen-Lawrence (2). The prolan of experiments V, VIII and IX was that of the Winthrop Chemical Company; of experiments VI and VII that of Squibb. Twenty-six day old male rats were used in all experiments excepting IX. These were distributed so that an equal division of litter mates occurred in each experimental group. In experiments I and II, each rat received one dose per day for four days and was sacrificed on the sixth day. In experiments III, V, VI, and VII one dose per day for 8 days was given, the rats being sacrificed on the 10th day. In experiment IV, one group of rats received 4 mgm. of powder per day for 8 days. Another group received each day divided doses of 1, 1 and 2 mgm. One milligram tannic acid was added to the last dose. In experiment VIII dosage was given over a period of 8 days. All groups received 5 units of prolan per rat per day. In addition two groups received 1 mgm. of pituitary powder per day; of these single daily dosage was used in one group and divided dosage (3 times per day) in the other group. It will be noted that the rats tolerated 1 mgm. tannic acid per day well, there being no appreciable loss in body weight. Higher doses produced body weight losses. The total dose of gonadotropic substance and tannic acid as well as the body weight and sex organ weights are given in table 1.

In experiment IX, 10 units prolan per rat was given in one dose to 6 males and 6 females 22 days of age. To 12 respective litter mates the same

amount of prolan was given over 4 days in 4 doses. The animals were sacrified the sixth day after initial dosage. The results are given in table 2.

TABLE 1

The effect of administering pituitary and urine of pregnancy gonadotropic extracts as the insoluble tannates

EXPT.	PERIOD DOSED	TOTAL DOSE GONADOTROPIC PREPARATION	TOTAL DOSE TANNIC ACID	DOSED DAILY	NO. RATS	WT. RATS	WT. SEMINAL VESICLES	P	TESTES	P
	days		*mgm.*			*gm.*	*mgm.*		*mgm.*	
I					6	60	9		426	
	4	4 mgm. pituitary		1	6	57	12		477	0.2
	4	4 mgm. pituitary	8	1	7	51	25	<0.01	531	0.01
II					6	54	9		381	
	4	20 mgm. pituitary		1	9	55	15	<0.01	465	0.04
	4	20 mgm. pituitary	16	1	7	41	34	<0.01	536	0.01
III					2	65	10		350	
	8	16 mgm. pituitary		1	4	57	12		381	
	8	16 mgm. pituitary	16	1	5	53	24	<0.01	538	0.2
IV										
	8	32 mgm. pituitary		1	4	70	24		706	
	8	32 mgm. pituitary	8	3	5	61	54	<0.01	837	<0.01
V					3	63	10		387	
	8	4.8 mgm. prolan		1	3	76	127		791	
	8	4.8 mgm. prolan	8	1	3	75	110		755	
VI	8	100 u prolan		1	6	65	44		552	
	8	100 u prolan	8	1	5	60	49	0.3	661	<0.01
VII	8	100 u prolan		1	5	60	88		810	
	8	100 u prolan	8	1	5	60	84	0.8	756	0.4
VIII	8	40 u prolan		1	7	72	49		603	
	8	40 u prolan 8 mgm. pituitary		1	6	73	43	0.05	658	0.01
	8	40 u prolan 8 mgm. pituitary		3	7	70	59	0.01	768	<0.01

ANALYSIS OF DATA. Since there is less variation in testicular weight among litter mates[1] than in the general population of the same age, the

[1] The correlation coefficient for the testicular weights of twenty pairs of litter mates in these studies was found to be 0.89.

significance of weight changes was tested out by comparing litter mate differences for groups. The formula for determining the significance of the mean of a unique sample as described by Fischer (3) was used. The values for P are given in the tables (comparison of litter mate values with those in the line above).

RESULTS. The addition of tannic acid to, or divided dosage of the pituitary preparation increased the effect upon the weight increment of the seminal vesicles in every experiment. In experiments I and III only slight increases in the weights of seminal vesicles or testes are indicated in animals dosed with the original powder. One hundred per cent increases in the weight of the seminal vesicles are indicated in the tannate-dosed animals. In experiments II and IV the weights of the seminal vesicles of the tannate-dosed animals are double those of the animals which received the original solution. In experiments I, II, III, and IV the testes of the tannate-dosed

TABLE 2

Comparison of the effect of administering the same total dose (10 u prolan) as a single dose or as four smaller doses (Exp. IX)

DOSAGE	NO. OF RATS	WT. OF RATS	WT. OF SEMINAL VESICLES	P	WT. OF TESTES	P	UTERINE HYPERTROPHY
		gm.	*mgm.*		*mgm.*		
Single	6	55	16		367		
Divided	6	53	30	<0.01	410	0.01	
			WT. OF OVARIES		VAGINA OPEN		
Single	6	47	22		3		3
Divided	6	47	25	0.1	1		6

rats weigh more (15 to 30 per cent) than those of the rats which received the original solution, and since P is 0.01 or less in three instances an augmentation effect upon testicular weight is demonstrated.

The addition of tannic acid to prolan (expts. V, VI, and VII) produced no augmentation in seminal vesicle weights. The testes of the tannate-dosed rats weigh more than those dosed with the original prolan in experiment VI. Such a difference is not revealed in experiment VII which was a duplication of experiment VI.

The addition of pituitary powder to prolan produced no augmentation effect upon seminal vesicles (expt. VIII). Divided dosage of prolan and pituitary powder increased the response over single dosage, but only to a degree corresponding to the effect produced by divided dosage of the pituitary preparation alone.

Whereas a series of daily injections of the prolan tannate (expts. V, VI,

VII) or a series of divided doses of prolan (expt. VIII) as compared with a series of single daily doses of prolan failed to show the augmentation effects which similar dosage procedures with pituitary preparations demonstrated, the more exaggerated condition, (expt. IX) in which the effect of one dose of prolan was compared with the effect of the same amount given in a series of daily doses, demonstrated an augmentation effect in increases in seminal vesicle and testicular weights. In females of the same age and under the same conditions of dosage the augmentation effect was of doubtful significance.

Discussion. The experiments show that delayed resorption (divided dosage) of pituitary gonadotropic preparations produces an augmentation effect on the sex organs of the immature male as was previously shown for the immature female rat. Doses which, given in the ordinary way, are minimal produce highly significant augmentation when given as the insoluble tannate. The pituitary preparation used was less effective in producing significant measurable changes in the male sex organs than in the female. In experiment II, single daily doses of 5 mgm. for 4 days produced a 50 per cent increase in the weight of the seminal vesicles, a 20 per cent increase in testicular weight. The same dose administered to female rats under the same conditions produced a 400 per cent increase in ovarian weight. It should be noted, however, that a 400 per cent increase in ovarian weight is no more on an absolute weight basis than a 20 per cent increase in testicular weight.

Maxwell (1) and Evans (4) state that divided dosage of prolan does not produce an augmentation of ovarian weight increases over single dosage. Dickens (5), however, has reported contrary findings. Our experiments show that the response to divided dosage of prolan can be demonstrated in males but only under exaggerated conditions. A division of dosage which would produce profound augmentation effects with the pituitary preparation fails to show augmentation with prolan.

SUMMARY

By administering pituitary gonadotropic extracts as the insoluble tannates or by dividing the dosage an augmentation in the weight increases of the seminal vesicles and testes of immature rats was produced.

When prolan was administered with tannic acid in repeated daily injections no augmentation in seminal vesicle weight increases was observed. However, by comparing the effect of a single dose with that of the same amount given in a series of daily doses an augmentation in weight increases was noted.

We are indebted to Miss M. L. Long for help in dosing and to the Winthrop Chemical Company for a supply of prolan.

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CHANGES IN THE PERMEABILITY OF RED CORPUSCLES IN SHED BLOOD TO GLUCOSE

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The author has recently found (Olmsted, 1935) that if blood is drawn from the ordinary laboratory mammals, including man, under proper conditions, there is little or no glucose in the corpuscles—the glucose is entirely contained in the plasma. The precautions to be observed are 1, the blood must be quickly drawn under oil; 2, no anticoagulant is to be added; 3, the blood is immediately to be centrifuged under oil without exposure to air in order to separate corpuscles and plasma at the earliest possible moment. The non-glucose fractions of the reducing substances are removed from the plasma thus obtained, and from whole blood by Somogyi's copper precipitation (Somogyi, 1931), and the glucose estimated by the Folin-Wu colorimetric method. Hematocrit readings are also made, and, having the glucose content of whole blood and plasma, the glucose content of the corpuscles can be obtained by subtraction.

In the dog, cat, rat rabbit, guinea pig and pig it was usually possible to show that there was no glucose in the corpuscles, but in man, no matter how carefully the procedure was carried out, a very small amount (5–15 mgm. per cent) of glucose appeared to be carried in the corpuscles. It was thought that this could be accounted for on the assumption that human corpuscles are less resistant to abnormal conditions of shed blood and that they might more rapidly lose their impermeability upon being removed from their natural environment than the corpuscles of the other mammals studied.

To show whether human corpuscles are less resistant than those of certain other mammals, blood was drawn under the precautions outlined above, and after samples were taken for glucose estimation just enough oxalate was added to the remaining whole blood to prevent coagulation. The changes in distribution of glucose between plasma and corpuscles were now followed for several hours at room temperature.

A typical set of results on man is shown in table 1. It will be noted that the increase in corpuscle glucose is very rapid at first, showing that the corpuscle rapidly loses its impermeability.

A typical set of results on rabbits' blood (table 2) may be contrasted with the results on human blood.

It will be seen that a greater proportion of glucose is to be found in the red corpuscles of man after 5 minutes than is found in rabbits' corpuscles under the same conditions after 2 hours.

At the opposite extreme from man is the pig. This animal was the most resistant of all the laboratory animals tried. Six hours after collecting

TABLE 1

Human blood

Milligrams glucose per 100 cc. Hematocrit 38 per cent

	PLASMA GLUCOSE	CORPUSCLE GLUCOSE	WHOLE BLOOD GLUCOSE	$\frac{\text{CORPUSCLE}}{\text{PLASMA}}$ RATIO
Without oxalate	140	30	77	0.21
5 min. after oxalate	98	42	77	0.43
10 min. after oxalate	91	52	76	0.57
30 min. after oxalate	86	58	75	0.68
1 hr. after oxalate	79	65	74	0.82
1½ hr. after oxalate	72	73	73	1.01

TABLE 2

Rabbits' blood

Milligrams glucose per 100 cc. Hematocrit 36 per cent

	PLASMA GLUCOSE	CORPUSCLE GLUCOSE	WHOLE BLOOD GLUCOSE	$\frac{\text{CORPUSCLE}}{\text{PLASMA}}$ RATIO
Before oxalate	151	0	97	0
4 min. after oxalate	148	6	97	0.04
15 min. after oxalate	140	14	95	0.10
30 min. after oxalate	136	19	94	0.14
1 hr. after oxalate	128	25	91	0.19
1½ hr. after oxalate	122	33	90	0.27
2 hr. after oxalate	114	42	88	0.37

the blood and adding oxalate to it, the glucose was found to be contained entirely in the plasma, none in the corpuscles.

CONCLUSION

These experiments show that the corpuscles of different mammals vary in their power to maintain their impermeability to glucose after the addition of oxalate. Human corpuscles rapidly become permeable, rabbits' corpuscles are more resistant, and pigs' corpuscles are quite resistant. This is strong evidence that the small amount of glucose the author has

hitherto found in human corpuscles is due to the manipulation of the blood, rather than to the presence of an appreciable amount of glucose in the corpuscles in the normal living body.

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PERIVASCULAR SEEP VALVES IN THE EXCHANGE OF BODY FLUIDS

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Under certain conditions, which will be discussed principally in connection with the schematic pen and ink drawings presented as figure 1, many lymph vessels and many blood capillaries and small veins are so permeable to water, whole blood and India ink that we must assume the presence of small openings or fenestrae in the walls of these vessels. The fenestrae are very schematically illustrated as small circular openings in A, C, D, E, F and G of figure 1. In B, the vessel is represented as being so greatly constricted that the fenestrae are only potential openings and that the walls of the vessel are accordingly very impermeable. The fenestrae become larger and the walls become increasingly permeable as the vessel dilates unless the dilatation becomes so great, as in C and at the ends of F and G, that the flow of liquid through the enlarged fenestrae is blocked by the severely compressed perivascular tissue. This tissue is a compression-decompression valve or a perivascular seep valve. Liquid seeps through it more readily when it is decompressed and accordingly rarefied, as in A, than when it is compressed and is accordingly dense, as in C.

Any compressible and decompressible tissue about a permeable vessel is the seep valve of that vessel, but it is convenient to speak somewhat arbitrarily of the valve as if it invariably consists of only a meshwork of connective tissue fibers exteriorly to the intima of any permeable blood or lymph capillary and exteriorly to the media of any permeable vein. In each of the schematic illustrations of figure 1, the seep valve is represented as consisting of only the cylindrical meshwork of connective tissue fibers in the perivascular space. Any permeable capillary lying within such a cylindrical valve consists of a very permeable layer of endothelial cells and often one or more Rouget cells, and any small vein found within such a cylindrical meshwork consists of a very permeable layer of endothelial cells (the tunica intima) and a slightly less permeable layer of muscle and connective tissue fibers (the tunica media). If it can be said that any of these vessels has a tunica adventitia or externa, this is not the characteristic layer of dense connective tissue external to the media of either a typical artery or a large vein but is only a relatively loose meshwork of

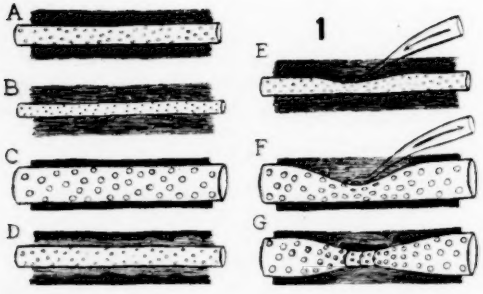

Fig. 1. These pen and ink drawings are some highly schematic representations of the conditions under which the permeability of a fenestrated blood or lymph vessel changes. The fenestrae are represented as circular openings when these are open and as dots when they are closed. The cylindrical seep valve is incompletely represented only above and below the vessel, so that the fenestrae and any mechanical derangement of either the vessel or the seep valve can be seen. The blackness of the valve indicates the state of compression and the state of impermeability of it.

A. The vessel is in a state of mild inflation. The seep valve is not strongly compressed and is accordingly somewhat permeable. The fenestrae are open. Liquid can pass into the vessel if the intervascular pressure is higher than the intravascular pressure or out of the vessel if the intravascular pressure is the higher.

B. The vessel is so severely constricted that the fenestrae are closed and that the vessel is impermeable although the seep valve is greatly decompressed and is accordingly very permeable.

C. The vessel is greatly inflated against the seep valve. The fenestrae are greatly enlarged, but the compressed valve is itself highly impermeable. The compressed valve also fits snugly over the fenestrae and closes them.

D. It must be imagined that the vessel has been quickly deflated after being strongly inflated. Some fibers of the innermost portion of the seep valve are attached to the walls of the vessel and are accordingly pulled inwardly as the vessel deflates. This proximal portion of the valve becomes quickly and greatly decompressed, but the distal portion exhibits an appreciable decompression lag. During this lag liquid flows rapidly into the rarefied portion of the valve and then into and through the distal portion as this also becomes rarefied.

E. A cannula is inserted into the perivascular space preparatory to urging liquid through the unbroken wall of a moderately dilated vein or lymph capillary. The arrow in the cannula indicates the direction of flow of the liquid.

F. This is the same as E except that the vessel is intentionally inflated so that the fluid passes from the vessel into the cannula at the only point at which the seep valve is rarefied. At all other points along the vessel the escape of liquid is blocked by the compressed valve.

G. This vessel, which must be imagined to be a blood capillary, is greatly inflated and is accordingly impermeable everywhere except in the immediate vicinity of the Rouget cell which holds a small portion of the capillary in a state of relative deflation.

connective tissue fibers in the perivascular space. Some of the fibers of the meshwork are connected with the walls of the vessel, but a much larger number of them are connected with the tissue exteriorly to the perivascular space.

The elasticity of the seep valve is less than that of the contained vessel. It is accordingly possible, after having greatly compressed the valve by strongly inflating the vessel (as represented by C of fig. 1), to deflate the vessel more rapidly than the outermost or distal part of the valve becomes rarefied (as represented by D of fig. 1). The innermost or proximal portion of the valve becomes greatly rarefied as the vessel deflates (D of fig. 1) because some of the fibers are attached to the vessel and are accordingly pulled inwardly as the walls of the vessel recede. If the vessel is inflated by injecting India ink into the network of vessels, none of the ink particles escape through the fenestrae as long as the ink pressure remains high and constant. However, upon reducing the injection pressure the ink escapes into and often through the seep valve, unless the reduction in the injection pressure is either very slight or very great. The ink escapes first into the rarefied, proximal portion and then into and often beyond the distal portion of the valve as this portion also becomes rarefied.

If the intravascular ink pressure is varied rhythmically and in such a manner that the conditions represented by C and D of figure 1 exist alternately, some ink flows out of the vessel every time the intravascular pressure is diminished and some of the escaped ink flows back into the vessel every time the injection pressure is increased. The outseep, however, is so much greater than the inseep that a considerable amount of the escaped ink is soon available for seeping into lymph vessels. Some of the escaped ink enters first the seep valves of some permeable lymph vessels and then appears in the lumina of these and other vessels of the lymphatic network (fig. 2). Moreover, some of the ink which seeps into the lymph vessels may seep out into the intervascular tissues again (fig. 3). Some of the ink may seep out of the lymph vessels at points quite remote from the points at which it seeps out of the blood vessels and then into the lymph vessels.

The superficial lymph vessels of an excised heart stand out in bold relief whenever the ink pressure in the blood vessels is quite high and the principal lymphatic trunks are occluded by tying off or clamping the cut tissues at the base of the heart. This inflation of the superficial lymph vessels is due principally to the passive decrease in the volume capacity of the deep lymph vessels of the network as these are compressed by the myocardium and the inflated blood vessels. As the injection pressure is increased, and the blood vessels accordingly undergo lateral and linear inflation, less room exists for the tissue fluid and also for the fluid that is already in the lymph vessels. Upon diminishing the ink pressure in the blood vessels, the superficial lymph vessels diminish in size because some of the lymph in them

flows back into the deeper lymph vessels among the temporarily deflated blood vessels. Some of this lymph even seeps back into the tissue spaces and carries some ink particles with it at the time that the seep valves about these lymph vessels are decompressed sufficiently that the perilymphatic tissue is itself quite permeable and that this tissue accordingly does not block the fenestrae in the walls of the lymph vessels.

Efforts to find the point or points of entrance of the ink into the lymph vessels of hearts were not always successful, but unmistakable evidence was obtained in some cases, by examining sections of cleared hearts, that this ink became available for entrance into the lymph vessels by escaping from ruptured blood capillaries. It is undoubtedly the case that some of the

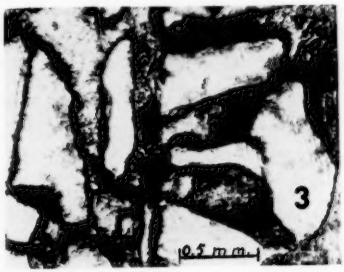

Fig. 2. These are mainly intraendocardial lymph capillaries from the left surface of the interventricular septum of the normal heart of a freshly killed lamb. These were injected through their unruptured walls by placing dilute India ink under considerable pressure in the left coronary artery and then varying this pressure rhythmically for many minutes. The "moth eaten" appearance of the vessels is due to ink which escaped through the walls of the lymph capillaries into the perilymphatic tissues when the pressure in the blood vessels was low.

Fig. 3. These lymph capillaries can be identified within the oval area marked off with pen and ink in figure 2. The ink which escaped through the walls of these vessels can be seen more clearly in this figure than in figure 2.

ink (which becomes available for filtration into the lymph vessels) merely seeps out of unruptured blood capillaries. Ruptured blood vessels could not be found in some of the hearts after the cardiac tissue was thoroughly cleared.

If some or all of the principal trunks of a lymphatic network are severed or otherwise opened peripherally to the lymph glands, ink that escapes from the blood vessels and into the perilymphatic spaces can rarely be urged into the lymph capillaries unless these vessels are artificially inflated by properly messaging the tissues or by injecting water into the network after the opened lymphatic trunks have been closed with ligatures. On the other hand, if no vessel of this network has been opened, ink soon

appears in considerable amounts in the lumina of many or all of the vessels of the network as the ink pressure in the blood vessels is rhythmically altered. Such observations suggest that each lymph gland normally offers a necessary resistance to the flow of the lymph. This resistance is great enough that the lymph capillaries rarely become either maximally deflated or maximally inflated. When the principal lymphatic trunks peripherally to the lymph glands are opened accidentally or intentionally, so much lymph escapes through the artificially created openings that the fenestrae in the walls of the lymph capillaries become only potential fenestrae, as illustrated by B of figure 1. If these potential fenestrae are converted into actual ones, by moderately inflating the vessels of the network, the available fluid in the perilymphatic spaces can then enter the lymph capillaries through the open fenestrae.

Some experiments show that the injection can be accomplished when the injection pressure in the perilymphatic spaces is very mild and when the rhythmic variations in the injection pressure are also mild. In one of these experiments, drops of a four per cent solution of sodium citrate were allowed to fall onto a small piece of cellophane which was placed over a part of an excised small intestine of an opossum. The cellophane was placed over a small ink hemorrhage in the intestinal wall. This hemorrhage was produced by first scraping the intima and media of a small artery with a slender quill-like bristle from a peccary and then mildly rupturing the artery at the point of the mechanical injury by injecting undiluted India ink into this vessel. After the artery was sufficiently ruptured to cause ink to become available for entrance into the lymph vessels, a cannula was inserted into it and then connected with a perfusion bottle which contained undiluted India ink. The hydrostatic pressure in the perilymphatic spaces amounted to about ten millimeters of the ink. The point of entrance of the bristle and also the point of insertion of the cannula was at a distance of about three centimeters from the point of rupture of the artery.

The artery was not ruptured until after the blood had coagulated in the arteries, capillaries and veins. Shortly after the coagulum formed, the tissue was moistened thoroughly with a four per cent solution of sodium citrate (to prevent the coagulation of the lymph) and the tissue was severely compressed at and near the cut margins (in order to retard the escape of fluid from the severed lymph vessels). The tissue was later kept moist by the non-coagulant as this dropped rhythmically onto the small piece of cellophane. By permitting the blood to coagulate before any ink was injected into the artery, the experiment was made desirably simple. The injected ink flowed between the coagulum and the walls of the relatively large arteries but not between this coagulum and the walls of the smallest arteries. For instance, the ink did not flow through the arteriocapillary anastomoses. The coagulum in the smallest parts of these

anastomoses, in the blood capillaries and in the veins did not become colored with the ink (fig. 4). Judging from results of similar experiments, some of the ink which escaped from the ruptured artery would have seeped into some of the blood capillaries and veins, as well as into the lymph capillaries, if the blood had not been coagulated in these vessels. The blood capillaries and veins cannot be seen in figure 4 because they and the coagulum in them became invisible when the specimen was cleared.

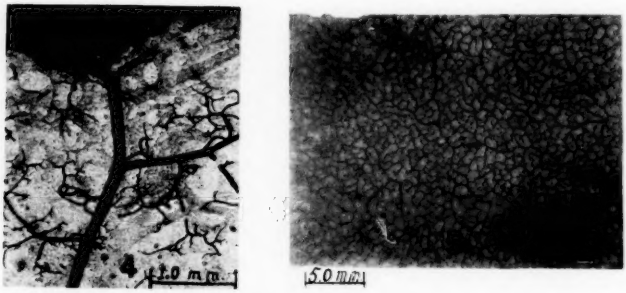

Fig. 4. Lymph vessels in a wall of the small intestine of an opossum. These were injected through their unbroken walls by mechanically rupturing an artery by injecting India ink into it after it had been scraped from the inside with a quill-like bristle of a peccary, and then permitting a four per cent solution of sodium citrate to drop onto the ink spot at the rate of one drop about every three seconds for a total time of about three hours and through a distance of seventy centimeters while the ink pressure in the perilymphatic tissues amounted to about ten millimeters of this fluid. In order to protect the tissue a piece of cellophane was placed over the black spot. A part of the black spot is shown in the upper part of the figure. The vessels in solid black are vessels injected with India ink. The blood capillaries and veins were not injected and are accordingly not visible in the photograph of the cleared specimen.

Fig. 5. This is a photograph of a part of the network of injected blood vessels in the uncleared white skin of the belly of a catfish. The network was injected, with dilute India ink, from a perivenous space by the method depicted in E of figure 1. Note the "moth eaten" appearance of the vessels at some points where the ink escaped into the perivascular spaces.

Microscopic examination of the specimen shows that none of the ink which seeped into the lymph vessels seeped out of them. The outseep of ink through the walls of such vessels does not occur unless the vessels themselves are somewhat strongly inflated and then deflated mildly at least once. Under the conditions of this experiment the lymph vessels did not become appreciably inflated and deflated at any time. This was the case although the periodic disturbance of the tissue underneath the small piece of cellophane was such that the black area at the point of the small rupture of the artery was about forty times larger at the end than at the beginning of the experiment.

An important feature of the experiment with the intestine was the mechanical injury and mild rupture of a normal artery in non-inflamed tissue. This point of technique is important because the greater number of my attempts to similarly inject lymph vessels in the vicinities of spontaneous ruptures were failures. This was almost invariably the case when the points of rupture of the blood vessels were in recognizably inflamed regions. On the other hand, when the ink is injected directly into the lymph vessels in the walls of an intestine the outseep of the ink occurs first and is generally greater in the inflamed areas than in the normal ones when the injection pressure is varied rhythmically. In some inflamed areas, only an outseep of the ink particles can be demonstrated. In some other inflamed areas a barely detectable inseep and a spectacular outseep of the ink can be demonstrated. In normal areas the ink seeps readily into the lymph vessels as well as out of them. The same is true of some blood capillaries and small veins in inflamed and normal areas of the intestine as well as of other parts of the body that I have investigated. These observations on the one-way seeping of ink in inflamed areas are fundamentally the same as some observations by Underhill (1930) on the one-way permeability of the walls of blood capillaries in burned areas.

My observations of tissue fluid from blisters of herpes febrilis and burns and from inflamed areas of intestine and other tissues have convinced me that extravascular cell débris is responsible for the greater outseep than inseep of liquid through the walls of the permeable lymph and blood vessels in such areas. This débris, like particles of India ink or similar débris artificially introduced into the perivascular space of a permeable vessel, drifts to the permeable vessels in the area and blocks the inseep of liquid by closing the fenestrae from the outside only. By varying the extravascular fluid pressure in a rhythmical manner, this cell débris (like the particles of India ink) can be sufficiently dislodged in many instances to permit the inseep of a considerable amount of liquid. Such débris should be spoken of as a perivascular drift valve or an outseep valve. It is conceivably an important function of phagocytes to ingest the cell débris and accordingly eliminate the outseep valve or the inseep hindrance which develops in inflamed areas.

The vessels shown photographically in figure 5 were injected by placing India ink under mild pressure in the perivascular space of one of the largest veins that could be seen (with a binocular microscope) through the white skin of the belly of a freshly killed catfish. Reference to E of figure 1 will help to make the injection method clear. While the small glass cannula was being inserted, it contained a small amount of water instead of ink. This water eliminated the possibility of my being concerned with air in the small end of the cannula and it allowed a clear field to work in while arranging the oval mouth of the cannula in the perivascular space. After

the cannula was properly inserted and fixed in a rigid position by means of a clamp on a heavy stand, a rubber tube was used to connect the large end of the cannula with a perfusion bottle that contained India ink. Upon elevating the bottle, the cannula and tube filled with ink as air escaped from them through the ink in the bottle. Air in such a perivascular space often seemed to compress the vessel without entering it.

While injecting the vessels of figure 5, an effort was made to regulate the elevation of the perfusion bottle so that the surface of the ink in it was exactly seventy millimeters above the mouth of the cannula during each of a series of five-minute injection periods. Every sixth minute was used to wash deposits of ink particles from the external surface of the media at the mouth of the cannula. This was done by raising and lowering the perfusion bottle rapidly and also in such a manner that the surface of the ink varied from about twenty millimeters above to about fifty millimeters below the mouth of the cannula. The injection and washing continued for two hours, but the vessels of the network were either perceptibly gray or entirely black almost everywhere after only fifteen minutes.

Attempts to inject the small vessels of other specimens by placing ink under a constant pressure in a perivascular space were not very successful unless the pressure was made so great that the ink broke through the tissues and entered the perivascular spaces of many other vessels of the network. When a constant, mild injection pressure was applied to ink in the perivascular space of a single vessel, the very permeable media and intima seemed to become rapidly less permeable, evidently because of deposits of ink particles in the passageways through these layers of tissue. Rhythmical variations in the injection pressure served to rhythmically dislodge many of the lodged ink particles and thereby repeatedly renewed the possibility of injection with a mild pressure. Briskly kneading or tapping the tissues of a bird or mammal in the region of a hypodermic injection of India ink probably serves this same purpose.

In some instances it was possible to demonstrate a perceptible flow of ink from a little vein of a catfish into the glass cannula when this cannula contained a small amount of water. After the cannula was properly inserted, a very dilute ink was injected with a hypodermic syringe into another small vessel at a distant part of the network. The needle of the syringe was inserted directly into the lumen of the vessel. When the filtration pressure, which was created by pressing on the plunger of the syringe, was later increased so enormously that the visible vessels of the body stood out in bold relief (as represented by C of fig. 1) the flow of ink into the cannula (inserted somewhat as in F of fig. 1) became quite rapid before the fluid began to escape through the opening in the skin through which the cannula had been inserted. As long as the intravenous pressure of ink was very high and the vessels were accordingly greatly distended, none of the ink escaped through the walls of any of these vessels except

where the perivascular tissue had been disturbed in the region of the mouth of the glass cannula. However, the ink escaped rapidly through the walls of many of the anastomoses of the network as soon as the injection pressure was diminished a little by removing the needle and permitting some of the ink to escape through the opening that had been made by the needle.

The same methods and some other similar ones were used to urge India ink through the unbroken walls of permeable veins and capillaries of many birds and mammals, but the results were rarely as spectacular as those obtained from the catfish.

It seems probable that the natural Rouget cell automatically regulates the natural pericapillary seep valve by contracting and relaxing in the ways that these cells are ordinarily believed to contract and relax. Independently of the Rouget cells, however, a capillary may become greatly constricted or greatly dilated. If it becomes greatly dilated while the Rouget cells are relaxed, the possibility of liquid escaping through the walls of the capillary and seeping through the condensed valve is reduced to a minimum. On the other hand, if the capillary is greatly dilated everywhere except at certain points where it is locally constricted as a result of the constriction by Rouget cells, intracapillary fluid can still escape through the capillary walls and through the locally decompressed valve. When the valve is compressed as a result of both capillary dilatation and inactivity of the Rouget cells, the fenestrae in the walls of the capillary are blocked and there is little or no seeping of liquid into the tissues beyond the perivascular space. The Rouget cells probably act as a safeguard against the failure of liquid to seep into the tissues when the capillaries are otherwise maximally dilated. It is conceivable that the Rouget cells normally act to permit intracapillary fluid to seep into the extravascular tissues from where at least some of it seeps into lymph capillaries.

If the little veins were constricted at intervals by narrow bands of tissue, many of the fenestrae in the immediate neighborhood of each band should remain open and the perivenous valve should be locally decompressed (as in G of fig. 1). Plasma should accordingly be able to pass out of the vein through the intima and media. Little veins of some fishes have such bands which consist of smooth muscle fibers and which probably act to cause local constrictions of the vessels (v. Brücke, 1925). Perhaps these local constrictors, like the Rouget cells, contribute to the maintenance of the normal water balance of the body.

In connection with the presumed function of the Rouget cells and the sphincters of little veins of certain fishes, it seems worth while to observe that veins of various sizes in diseased areas of birds and mammals often show local constrictions. I have seen such local constrictions of small meandering veins in vascularized, inflamed corneas of living animals.

If the seep valve functions in the ways herein described, it would seem

well to modify our usual ways of thinking about some things. For instance, if the blood that is lost during a rapid, severe hemorrhage is replaced by a liquid of low viscosity, the lateral pressure of this liquid on the walls of the capillaries and small veins may not suffice to compress the cylindrical valves sufficiently to prevent a rapid seeping of the liquid into the tissues, even if no other factor (as osmosis) were involved. A viscous liquid, such as a six per cent or a ten per cent solution of gum of acacia, flows so sluggishly in the little vessels that the lateral pressure is great enough to compress the perivascular valves and other extravascular tissues and thereby prevent a rapid seeping of the liquid from the capillaries and little veins. However, while the viscous fluid is in the vessels, liquid can seep into the capillaries and little veins from the tissues whenever the pressure of the tissue fluid exceeds the lateral pressure in the vessels sufficiently to mechanically constrict them a little and accordingly decompress the perivascular valve.

The colloids of normal blood should act similarly as the gum of acacia. By virtue of their lateral pressures they should keep themselves within the vessels to a considerable extent and incidentally keep up a certain osmotic pressure of the blood.

SUMMARY AND CONCLUSIONS

1. The permeability of the walls of numerous lymph capillaries, blood capillaries and small blood veins is mechanically regulated by a cylindrical meshwork of connective tissue fibers in the perivascular space of each of these vessels. This meshwork functions as a seep valve. It can also be called the cylindrical valve, the perivascular valve or the compression-decompression valve.

2. When the valve is maximally compressed, as when a vessel in normal tissue is maximally dilated (as a result of the lateral pressure of a viscid, sluggishly flowing, colloidal solution, mere inactivity of the vessel walls or a greater intravascular than extravascular pressure of fluid), very little or no intravascular liquid seeps into the tissues because the compressed valve is itself very impermeable and because it snugly closes fenestrae in the walls of the vessel.

3. On the other hand, if a vessel constricts (as a result of a decrease in the viscosity of the circulating fluid, a relatively high extravascular fluid pressure or an active constriction of the vessel), the cylindrical meshwork opens up, accordion-like, and functions as a decompression valve. The decompressed or rarefied valve no longer snugly closes the fenestrae in the walls of the vessel and it is itself relatively permeable. Whether liquid then seeps into or out of the vessel depends upon whether the liquid pressure is the higher in the vessel or in the extravascular tissues.

4. If a capillary is maximally dilated except where it is locally con-

stricted by a Rouget cell, the perivascular cylinder of connective tissue fibers (the seep valve) functions as a compression valve at all points of maximal dilatation and as a decompression valve where the connective tissue is rarefied in the vicinity of the Rouget cell.

5. Rhythmic variations in the intravascular and intervascular fluid pressures contribute considerably to the flow of liquid back and forth through the walls of the permeable lymph and blood vessels.

6. Perivascular cell débris in inflamed areas, like particles of India ink introduced into perivascular spaces in normal tissues, drifts to the walls of permeable vessels and prevents the inseep of liquid by closing the fenestrae from the outside only. The cell débris, like the India ink, accordingly forms a one-way seep valve. This is an outseep valve which functions somewhat imperfectly if the débris is rhythmically dislodged from the walls of the vessels by varying the extravascular fluid pressure in a rhythmical manner.

7. The lymph gland offers enough resistance to the flow of lymph that the lymph capillaries peripherally to the gland are in a sufficient state of lateral inflation that tissue fluid can readily seep into them. If the resistance (the lymph gland) is eliminated, the amount of liquid that seeps into the lymph capillaries is greatly reduced.

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VALUES FOR NUMBER, SIZE AND HEMOGLOBIN CONTENT OF ERYTHROCYTES IN NORMAL DOGS, RABBITS AND RATS[1]

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In the course of certain experimental studies, observations on the blood of 54 normal adult dogs, 61 rabbits and 73 rats were carried out. Since there is relatively little information in the literature concerning "normal" blood values in these animals, our findings are briefly recorded.

MATERIAL AND METHODS. The *dogs* were adult mongrels of various ages and weighed 5 to 17 kgm. They were maintained in separate cages on a diet consisting of boiled beef lungs, white wheat bread and water and this was occasionally supplemented with cod liver oil. The dogs were kept in the laboratory for several weeks before any blood examinations were made and appeared to be in good condition.

The *rabbits* were of mixed types, chiefly brown and gray in color, with some white or black. They were 6 to 9 months of age, and weighed 1600 to 3200 grams. They were usually caged separately and were fed a diet of whole wheat, oats, hay and water.

The *rats*, 6 to 12 months of age, were of an inbred mixed stock, chiefly white with some black and tan. Their diet was made up of corn (2280 gm.), linseed (480 gm.), casein (150 gm.), calcium (15 gm.), salt (15 gm.), alfalfa (60 gm.) and powdered milk (1000 gm.).

Blood was obtained from the dogs by puncture of the jugular veins and was collected in heparin or in a mixture of ammonium and potassium oxalate. It was found that a mixture of 1.3 mgm. of dry ammonium oxalate and 0.7 mgm. of dry potassium oxalate per 1 cc. of blood is an effective anticoagulant and causes no alteration in the size of the red corpuscles of dogs, rats or rabbits.

Blood from the rabbits was collected in heparin or in the above oxalate mixture by allowing it to drop from the punctured ear vein into a bottle containing the anticoagulant.

All the blood samples in the rats were obtained by cardiac puncture. The above mentioned anticoagulants were used.

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Red cell counts, hemoglobin and volume of packed red cells were determined and from these data the mean corpuscular volume and hemoglobin content were calculated (1). At least two red cell counts were carried out in each instance and when these did not check within 100,000 cells per c.mm. they were repeated until checks were obtained. Hemoglobin was determined in a restandardized Newcomer hemoglobinometer and the volume of packed red cells was determined in a special hematocrit (2).

Blood was withdrawn usually between the hours of 9 and 11 a.m. The majority of the examinations were made between the months of September and June but some observations were carried out during the summer months.

Observations. The results of these blood examinations are recorded in figure 1. The data for mean, standard deviation (S. D.), and coefficient of variation (C. Va.) have been calculated from 173 blood examinations in 54 dogs, 168 blood examinations in 61 rabbits, and 109 determinations in 73 rats. In this figure C. V. refers to the mean volume of the red corpuscles (mean corpuscular volume) measured in cubic microns, C. H. to the mean weight of hemoglobin in each red cell (mean corpuscular hemoglobin) measured in micromicrograms, and C. C. to the proportion of the volume of the average red corpuscle made up by hemoglobin (mean corpuscular hemoglobin concentration) expressed in per cent.

The histograms for red cell count, hemoglobin and volume of packed red corpuscles are fairly symmetrical but those for mean corpuscular volume in the rabbits and in the rats, and for mean corpuscular hemoglobin in the rats are quite irregular. For this we have no explanation.

Discussion. In table 1 reports of erythrocyte studies in all series of 10 or more normal dogs which we have been able to find are summarized. It may be pointed out that the values for mean corpuscular volume and hemoglobin concentration calculated from the data of Powers, Bowie and Howard (6) cannot be considered correct since no allowance was made by them for shrinkage of red corpuscles caused by the anticoagulant used. Mayerson's (7) very low value for mean corpuscular volume is also open to question because he employed the Van Allen hematocrit, an instrument in which leakage is very difficult to prevent.

We have found no reports of erythrocyte studies in any large series of rats and only the papers of Bushnell and Bangs (9), Jackson and Stovall (10), Pearce and Casey (11) and Ashley and Guest (13) concerning counts in any large groups of rabbits. The first mentioned investigators recorded only red cell counts while Jackson and Stovall and Pearce and Casey gave only erythrocyte counts and hemoglobin. The hemoglobin values recorded by Jackson and Stovall are worthless, moreover, since the method of hemoglobin determination they used (Tallquist) is unreliable.

Our observations on red cell counts in rabbits agree more nearly with

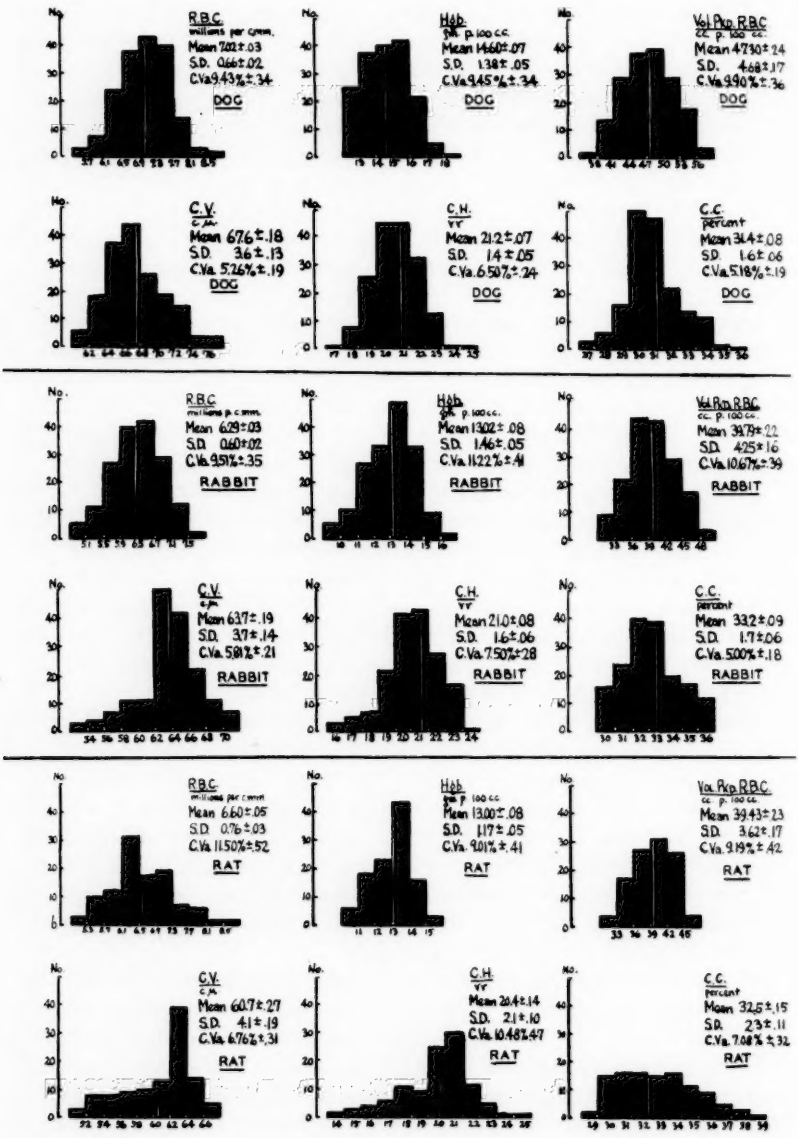

Fig. 1. Results of blood examinations in dogs, rabbits and rats. For description, see text.

those of Bushnell and Bangs and of Ashley and Guest, who found averages of 5.99 and 6.41 million cells per c.mm. in 100 and in 55 rabbits respectively, than with those of Jackson and Stovall or Pearce and Casey who reported counts of 5.31 and 5.20 millions in 15 and in 174 rabbits respectively. Pearce and Casey found the mean hemoglobin of their rabbits to be 63 per cent but did not give the equivalent in grams. Ashley and Guest found the average hemoglobin to be 12.8 grams, the volume of packed red cells, 37.7 cc., the mean corpuscular volume 68.1 c. μ, mean corpuscular hemoglobin 23.2 micromicrograms and mean corpuscular hemoglobin concentration 34.0 per cent.

In man, during adult life, a well marked difference between the sexes as regards red cell count, hemoglobin and volume of packed red cells is found.

TABLE 1

Summary of reported studies on normal dogs

INVESTIGATOR	NO. OF ANIMALS	R.B.C. × 10^6	HEMOGLOBIN		VOL. PACKED R.B.C.		C.V.	C.H.	C.C.
			Grams	Instrument	Cc.	Anticoagulant			
							$c.\mu$	$\gamma\gamma$	*per cent*
Dawson (3)	15	7.22	11.9	v. Fleischl				26.0	
Musser and Krumbhaar (4)	47	5.97	15.5	v. Fleischl					
Stucky and Rose (5)	12		15.0	Cohen-Smith					
Powers, Bowie and Howard (6)	25	7.00	15.8*	Sahli	45.2**	16 mgm. dried Na oxalate per 5 cc. blood	64.5	23.6	35.0
Mayerson (7)	60	6.49‡	13.0	Newcomer-V.S.	38.6	1.6% Na oxalate solution	59.3	20.0	34.3
Leichsenring et al. (8)	32	7.17	14.1	Van Slyke	47.7	Not stated	66.5	19.7	29.6
Ashley and Guest (13)	50	6.87	16.0	Van Slyke and Hiller	45.6	Heparin	66.6	23.6	34.9
Wintrobe, Shumacker and Schmidt	54	7.02	14.6	Newcomer-V.S.	47.3	See text	67.6	21.2	31.4

* Estimated. Hemoglobin recorded as "92.8 per cent Sahli" by authors.

** No correction made for shrinkage in volume caused by anticoagulant.

‡ Calculated from author's table 1.

The cause of this difference is not known, nor, as far as we are aware, has it been clearly demonstrated that such a difference between the sexes exists in other mammals. One possible explanation for the difference encountered in man is the monthly loss of blood by the female. If this factor is of significance, one would expect that in species of mammal in which there is no monthly loss of blood, little or no difference between the blood counts of the sexes would be found.

In table 2 our own observations in male and female dogs, rabbits and rats are recorded and the data available in the literature are also presented. The exact agreement of the findings in the two sexes is quite striking. Only in the dogs we examined and in the rabbits studied by Rosahn et al. (12) were the blood counts slightly lower in the females than in the males.

The studies of Rosahn et al., however, constitute an important exception because their series is the largest of all that have heretofore been recorded and the male and female rabbits they examined were litter mates and not chosen at random as were the animals of all the other groups studied. Although the differences they noted were small, Rosahn and his co-workers concluded that they were statistically significant. No conclusions are therefore offered regarding the comparison of the blood of male and female dogs, rabbits or rats but the data are presented in the hope that further studies may be recorded.

TABLE 2

Comparison of blood counts in male and female animals

ANIMAL	INVESTIGATOR	NO. OF ANIMALS AND SEX	R.B.C. $\times 10^6$	Hb.	Ht.
				grams	*cc.*
Dog	Mayerson (7)	37 male	6.47	12.9	37.6
	Mayerson (7)	23 female	6.53	13.2	39.7
Dog	Leichsenring et al. (8)	16 male	7.17	14.1	48.0
	Leichsenring et al. (8)	16 female	7.17	14.1	47.5
Dog	Wintrobe et al.	22 male	7.16	15.1	48.7
	Wintrobe et al.	10 female	6.78	13.9	44.7
Rabbit	Rosahn et al. (11)	41 male	5.64	72%	
	Rosahn et al. (11)	41 female	5.34	67%	
Rabbit	Guest (14)	32 male	5.89	12.9	38.9
	Guest (14)	30 female	5.60	13.0	38.6
Rabbit	Wintrobe et al.	35 male	6.25	12.9	39.4
	Wintrobe et al.	26 female	6.30	13.4	40.1
Rat	Wintrobe et al.	47 male	6.61	13.0	38.0
	Wintrobe et al.	26 female	6.45	13.3	40.4

SUMMARY

1. The erythrocyte count, hemoglobin, volume of packed red cells and mean corpuscular volume, hemoglobin and hemoglobin concentration of 54 normal adult dogs, 61 rabbits and 73 rats are recorded (fig. 1).

2. The reports on this subject which have been found in the literature are summarized.

3. The blood of male and female dogs, rabbits and rats is compared (table 2).

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EFFECT OF OESTRIN ON THE ACTIVITY OF THE ANTERIOR LOBE OF THE PITUITARY[1]

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Several investigators have studied the effects of oestrin on the activity of the pituitary and on ovarian growth and development. It has been found that oestrin decreases the potency of the pituitary as measured by transplantation of the pituitaries of oestrin treated rats into immature females (Meyer et al. 1930 and 1932, Wolfe 1935). Hohlweg (1934), and Selye (1935) found that in mature rats oestrin causes excessive luteinization of the ovaries. Hohlweg found, however, that oestrin caused no increase in size and no luteinization in the ovaries of immature rats (1934).

None of the methods employed so far give a clear picture of the physiological changes which take place in the pituitary and the ovaries under the influence of oestrin. The transplantation method of testing pituitary potency measures the combined activity of the follicle stimulating hormone (F.S.H.) and the luteinizing hormone (L.H.) of the pituitary. Since these two act synergistically to produce the ovarian response (Fevold et al., 1934a), this method cannot be used as an exact measure of either the F.S.H. or the L.H. Also, it does not measure the activity going on in the pituitary but merely the condition to which the pituitary is brought by the treatment.

In an attempt to obtain more accurate information concerning the effect of oestrin on the secretory activity of the pituitary with regard to F.S.H. and L.H., we devised experiments to measure the relative amounts of each (Fevold et al., 1934b). To do this we made use of the augmentation reaction between F.S.H. and L.H. and between F.S.H. and the hormone from pregnancy urine (P.U.) based on the following considerations. In a normal immature rat only sub-minimal amounts of L.H. are secreted as indicated by the fact that luteinization usually does not take place when F.S.H. is administered and also by the fact that the action of F.S.H. in an immature rat is limited in its ability to increase the weight of the ovaries.[2] If oestrin increases the amount of L.H. secreted by the pituitary this should

[1] Assisted by grants from the National Research Council, Committee for Research in Problems of Sex and the Wisconsin Alumni Research Foundation.

[2] Unpublished data.

lead to augmentation of the ovarian weight when F.S.H. is administered and the ovaries may be luteinized. Similarly an increase or a decrease in the output of F.S.H. should be reflected in the ovarian response to the injection of P.U. since it acts synergistically with F.S.H.

EXPERIMENTAL. The first group of experiments reported below were carried out in the following manner. Twenty-one day old rats were injected for three days, twice daily, with varying amounts of F.S.H. or P.U. They were then killed 24 hours after the last injection and the ovaries weighed and examined. The data presented in the tables are the results of final experiments set up to illustrate the paper. The weights of the ovaries are the averages of six rats in each group.

Table 1 presents results obtained by injecting various amounts of oestrin followed by F.S.H. The results demonstrate clearly that the ovarian reaction produced by F.S.H. injections has been materially altered by the previous treatment with oestrin. The ovaries are much larger and are

TABLE 1

*Effect of oestrin on the ovarian response to F.S.H.**

	NORMAL RATS							HYPOPHYSECTOMIZED RATS	
Oestrin R.U. (daily).....	None	0.1	0.2	0.5	1.0	2.0	4	None	0.5
Weight of ovary (mgm.).....	24	32	46	73	62	53	61	29	30
Structure of ovaries	F	F-C.L.	C.L.	C.L.	C.L.	C.L.	C.L.	F‡	F

* All rats received 0.25 mgm. F.S.H. except hypophysectomized rats which received 0.4 mgm. F = Follicle. C.L. = Corpora lutea.

heavily luteinized in every instance except in the group which received 0.1 R.U. per day. Here the results were variable, the ovaries of some being quite well luteinized while others were almost free of lutein tissue. We may say then that 0.1 R.U. daily is the lower limit for stimulation under these experimental conditions. One-half R.U. per day seems to be the optimum for stimulation and any further increase in dosage of oestrin gives no greater response.

The last two columns of table 1 give the results of two groups of hypophysectomized rats which served as controls. They were hypophysectomized when they were 25 days old and the oestrin injections were started one day later. No difference was found in the ovarian response of the oestrin-treated from that of non-oestrin treated rats. The ovaries of both groups were of the same weight and were composed of follicles with no luteinization.

Table 2 gives the results obtained when P.U. was substituted for F.S.H.,

the experiments being otherwise carried out as before. It was found that the pretreatment with oestrin had no noticeable quantitative or qualitative effect on the ovarian response. Controls in the same group in which the oestrin treatment was followed with F.S.H. showed good augmentation and luteinization.

In a second group of experiments the rats (21 days old) were given varying amounts of oestrin for eight days followed by the usual three day injections of F.S.H. or P.U. (tables 3 and 4). After they had been treated for this length of time the response to F.S.H. was different from that obtained after a three day oestrin treatment. No augmentation of F.S.H. action occurred but instead, with increasing oestrin dosage, the ovarian response to F.S.H. was progressively reduced. In fact, 4 R.U. of oestrin daily reduced the expected F.S.H. effect 400 per cent. In addition the follicles are not

TABLE 2

Effect of oestrin on the ovarian response to P.U.

	1 MGM. P.U.			CONTROLS 0.25 MGM. F.S.H.	
Oestrin R.U. daily	None	0.5	1.0	None	0.5
Weight of ovary mgm.	30	28	29.0	22	80

TABLE 3

Effect of an eight day oestrin treatment on the ovarian response to F.S.H. (0.25 mgm.)

Oestrin R.U. daily	None	0.1	0.5	1	4
Weight of ovary mgm.	30	24	23	23	17
Structure of ovary	F	F	F	F + CL	F

luteinized in these experiments as was true when oestrin was injected for three days previous to F.S.H. injections.

Similar results were observed when P.U. was given after an eight day oestrin treatment. With increasing oestrin dosage the ovarian response to P.U. also decreases regularly.

Since the quantitative effect of eight day injections of oestrin on the response to F.S.H. and P.U. are quite similar the question naturally came up as to whether oestrin may not have a direct effect on the ovary, reducing its ability to respond. To test this, hypophysectomized rats were used in experiments similar to those related above. Two groups of rats, which were hypophysectomized when they were 28 days old, received 0.1 and 0.5 R.U. of oestrin daily for eight days followed by F.S.H. while the controls were untreated for the same period after which they also were given the same dosage of F.S.H. (table 5). The results, however, were the same

in both groups of animals showing that the ovarian response was not altered in the least by previous oestrin treatment.

DISCUSSION. From the results presented in this paper it seems that the first effect of oestrin on the pituitaries of immature rats is to increase the secretion of L.H. This is shown by augmentation of the action of the F.S.H. which is injected during that period. The fact that Hohlweg obtained no increase in ovarian weight in sexually immature rats with oestrin treatment alone does not indicate that L.H. was not secreted as it has been shown that L.H. by itself does not increase the size of the ovaries in immature rats (Fevold et al., 1933). There is no indication in these experiments that the secretion of F.S.H. is stimulated during oestrin treatment as the action of P.U. is not augmented. Consequently in Hohlweg's experiments the conditions would be the same as if L.H. were injected alone.

TABLE 4

Effect of an eight day oestrin treatment on the ovarian response to P.U. (1 mgm.)

Oestrin R.U. daily	None	0.1	0.5	1	4
Weight of ovary	29	24	22	20	18
Structure of ovary	CL + F	CL + F	CL + F	CL + F	CL + F

TABLE 5

Effect of oestrin on the ovarian response of hypophysectomized rats to F.S.H. dosage (1.0 mgm.)

Oestrin R.U. daily (7 days)	None	0.1	0.5
Weight of ovary	46	45	50
Structure of ovary	F	F	F

When oestrin is injected for longer periods (8 days) the results are quite different in that the ovarian response to F.S.H. or P.U. is decreased. That this decrease is not caused by the direct action of oestrin on the ovaries is shown by the lack of any effect of oestrin on the ovarian response to F.S.H. in hypophysectomized animals. It is evident that the pituitary is involved in the reaction but at the present time we do not know how the decrease in response is brought about. It may be due to exhaustion of the pituitary with consequent elimination of the pituitary secretions (F.S.H. and L.H.) which normally act synergistically with the injected substances or it may be due to partial involution of the ovaries following cessation of pituitary secretion. More work is necessary to prove or disprove these theories.

The data presented here explain the fact that long treatment (8–10 days) of rats with F.S.H. eventually results in luteinization of the ovaries. The follicles produced by the F.S.H. secrete oestrin as shown by the opening of the vaginal orifice and the enlarged uterus. The oestrin would likewise

affect the pituitary in the same manner as the injected follicular hormone, causing it to secrete luteinizing hormone.

In the rat, at least, the effect of the follicular hormone on the pituitary may be an important step in the regulation of the oestrous cycle. It is, of course, quite commonly recognized that the pituitaries of castrated rats are more potent and those of oestrin treated rats less potent than those of normal animals when measured by transplantation. When the follicles of a rat's ovary are developed and secrete the follicular hormone, it may act on the pituitary to decrease its follicle stimulating ability and increase the output of luteinizing hormone. The L.H. then luteinizes the follicles, cuts off the follicular hormone and allows the F.S.H. to again increase and the process is repeated.

In this connection it is significant to point out that "Prolan A," the follicular stimulating hormone from urine, is found in the urines of individuals whose source of follicular hormone or its male equivalent, the male hormone, is absent or at a very low level. Thus it has been found in urine from castrated women, women past the menopause (Zondek, 1930), and castrated men (Hamberger). Hellbaum (1933) has also found that the pituitaries of castrated horses contain large amounts of F.S.H. but very little L.H. It seems possible that the absence of oestrin or male hormone may account for the absence or the scantiness of luteinizing hormone.

SUMMARY

The first effect of oestrin on the anterior lobe of the pituitary is to increase the secretion of luteinizing hormone. This opinion is based on the fact that oestrin treatment augments the effects of a given dosage of follicle stimulating hormone as shown by an increase in weight of the ovary and luteinization. This action of oestrin is by way of the pituitary, as augmentation of follicle stimulating hormone by oestrin treatment does not occur in hypophysectomized animals.

Oestrin treatment does not augment the action of pregnancy urine, signifying that the secretion of follicle stimulating hormone is not increased.

When oestrin is given for an extended period (8 days) the response of the ovary to follicle stimulating hormone and pregnancy urine is greatly decreased. This may be due to excessive stimulation of the pituitary by oestrin resulting in secretory exhaustion and consequently partial involution of the ovaries.

The relation of oestrin to the secretion of follicle stimulating and luteinizing hormones by the pituitary may be important for the regulatory mechanism of the oestrous cycle.

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